THE DEVELOPMENT

of

A DYE-DILUTION TECHNIQUE

for

THE ACCURATE ESTIMATION OF CARDIAC OUTPUT

and

A CLINICAL APPLICATION OF THE TECHNIQUE

by

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INTRODUCTION

HISTORICAL SURVEY OF THE DEVELOPMENT OF INDICATOR-DILUTION TECHNIQUES

General Survey

"De motu cordis et sanguinis in animalibus" by William Harvey appeared in 1628, and contained the first documented quantitative method used in cardiovascular physiology. Harvey estimated the stroke volume of the left ventricle at two to three ounces, and the cardiac output as ranging from 10 - 41 pounds of blood per half hour in man (Franklin translation, 1957). Stephen Hales (1733) made wax casts of the distended ventricles of the horse, and, assuming that the ventricle emptied with each stroke, he multiplied left ventricular volume by heart rate to obtain the rather small figure for cardiac output of six litres per minute. Despite having made no allowance for residual volume, his underestimation of cardiac output may have been partly due to the heart having been in rigor mortis when the wax was poured in.

These two instances were the earliest attempts to measure cardiac output. The development of the indicator-dilution technique ran along somewhat different channels, and developed from methods used for the study of circulation times. Haller (cited by Stewart, 1893), in 1761, injected a coloured liquid into the vena cava of a freshly-killed animal, to compare pulmonary circulation times through the inflated and collapsed lungs. In 1827 Hering (quoted by Tigerstedt, 1921) injected potassium ferrocyanide into the jugular vein of a horse, and sampled from the opposite jugular, the time of indicator arrival being determined by testing successive samples for the Prussian blue reaction by the addition of ferric chloride. From this he measured what he called the circulation time, which was in fact the fastest circulation time, or appearance time.

These early studies illustrated the growing need for a discontinuous sampling technique, which was fulfilled by two notable advances. Vierordt (quoted by Blumgart and Yens, 1927) arranged a number of cups on a revolving disc below a blood vessel, from which blood was to be sampled. The rate of disc rotation was known, so that it was possible to measure circulation time with considerable accuracy. Hermann (quoted by Stewart, 1918) allowed blood to play on a revolving drum covered with paper soaked in ferric chloride, after injection of potassium ferrocyanide; yet another advance.

In 1870, Fick expounded the theory of a dilution principle as a means of measuring blood flow, a method not employed in practice until the studies of Grehant and Quinquaud, and Zuntz and Hagemann in 1886 and 1898 respectively. It is doubtful, however, whether this principle sparked the mind of Stewart to develop the first injection method for measuring cardiac output in 1897, although the Fick principle was in fact a specific example of a dilution method in which oxygen is the physiologically-added indicator. The same principle is involved if a foreign substance is introduced into the bloodstream, either by injection or inhalation, or if a substance is removed from, or added to the bloodstream at a known rate by the function of the liver or kidneys.

Stewart's historic use of sodium chloride solution as an indicator in the measurement of cardiac output (1897, 1921 a, b) undoubtedly stemmed from his earlier work in 1893 on circulation times through organs, performed at Edinburgh University, the results of which he submitted as part of a thesis for the Goodsir Memorial Prize in the University of Edinburgh in 1892. A solution of hypertonic sodium chloride was introduced into the arterial supply

of the organ, a vessel draining it isolated, and placed over two nonpolarizable electrodes, so that the blood vessel formed one arm of a Wheatstone bridge. The galvanometer of the bridge was replaced by a telephone, and the bridge balanced to yield minimum noise. On arrival of the injectate at the outflow vessels, the bridge was unbalanced, and the telephone note changed. The time between the injection and the change in sound represented the organ circulation time. Where a vessel was too small to be manipulated over the electrodes, he substituted methylene blue as an indicator, and was able to detect its arrival by a change in colour seen through the transilluminated blood vessel wall (Stewart, 1918).

Subsequently, Stewart went on to measure the concentration of the indicator quantitatively, and from his results derive cardiac output and the volume of blood between the sites of injection and sampling (1897, 1921 a, b). In these experiments, he isolated both femoral arteries and to the one connected his electrodes, while the other he used as a source of indicator-blood mixture. A sodium chloride solution of known composition was run in for a known number of seconds through a tube passed into the dog's superior vena cava, right atrium or left ventricle. When the telephone note announced the arrival of the indicator wave, a bulldog clamp was released on the opposite femoral artery, and an indicator-blood mixture sample collected. The calibration consisted of diluting a sample of control blood, taken before the sodium chloride injection, with a measured quantity of the indicator solution, until the blood-salt mixture equalled the conductivity of the arterial sample, the salt concentration of which could thus be derived. The concentration, volume and rate of injection of the indicator were known and the final concentration of indicator in blood was achieved because the injectate was diluted by the flow.

To some extent, Stewart employed a semi-instantaneous injection technique, and sampled for a known period of time, including all the indicator-blood mixture as it passed the sampling site. Although this method did not conform to the present-day criteria of an instantaneous injection, with this sustained injection technique, Stewart described the basis of what has become the instantaneous injection and the constant infusion techniques of measuring flow by the indicator-dilution method.

Henriques (1913) was the first to pursue the subject after Stewart. Appreciating the possible loss of indicator in the lungs, he injected instantaneously into the left ventricle or aorta, using as an indicator sodium thiocyanate because of its ready colorimetric estimation, and employing a rapid multiple sampling technique. He was the first to remark on the error caused by indicator recirculation, showing quite clearly that the concentration never returned quite to zero, and also the presence of a recirculation peak. To avoid this error he limited his sampling period to twelve seconds after injection, and used an integrated sampling method.

Bock and Buchholtz (1920) used Stewart's constant infusion technique with sodium iodide as an indicator, which they injected at a constant rate from a gear-driven metal syringe, to ensure an absolutely constant infusion rate. They also showed that a secondary rise of concentration could occur from recirculation with too long an infusion time.

An explosive article from Stewart followed (1921), in which he openly attacked Henriques, and Bock and Buchholtz on several accounts. Opinions differ (Dow, 1956; Fox, 1962) on the justification for his comments. While Henriques, and Bock and Buchholtz did contribute considerably to the

original concept, as Stewart rightly pointed out, the fact that they changed the nature of the indicator did not mean that theirs was a new concept. It is of interest to quote some of Stewart's rejoinders concerning a technique which was to have a stormy passage before its eventual universal acceptance:

"In my opinion however, Henriques, and Bock and Buchholtz have lost something of value in not being able to know the actual time of arrival at the point of collection of the substance injected by them until after the experiment, so that they have to guess at the proper time to collect their samples. This would be a drawback especially in studying the output under conditions where considerable changes were occurring in the velocity of the bloodstream. Bock and Buchholtz employed the first of the two procedures discussed above, determination of the maximum concentration of the injected substance and the rate at which it is injected. They seem to consider that their experiments were done according to a somewhat different principle than mine . . . . the fact is that Bock and Buchholtz used the principle of our first procedure exactly, while employing a different substance (sodium iodide) for injection, while Henriques employed our second procedure, but with sodium sulphocyanide. Both of these substances are stated to be harmless in the quantities employed, but they cannot possibly be superior to a weak sodium chloride solution in this respect".

This paper has sparked off further controversy between Dow (1956) and Fox (1962) whether Stewart truly appreciated the possibilities of an instantaneous injection, or the dispersion effect of the circulation on such an injectate, before the contributions of Henriques, and Bock and Buchholtz.

It seems probable that he did in fact realise the dispersion effect, as evidenced by his preoccupation with the variation between the fastest and mean circulation times of indicator, its repeated change from axial to peripheral streams, as well as his statement:

"There is always a certain thinning out of the column (of injected saline) at its front and rear, as can be well shown by the somewhat gradual increase and decline of the sound in the telephone".

He was well aware of the effect of laminar flow on indicator dispersion, and demonstrated in model experiments that the fastest traversal time of indicator was somewhat greater than half the traversal time calculated by the "flow x volume" formula that would pertain if the mixture of dye and blood maintained a square wave front during traversal of a tube (Stewart, 1897).

As Fox (1962) points out, Stewart was aware of both constant infusion and instantaneous injection techniques, although in his original article he did not stress their inherent differences. Although he justifiably criticised the other authors for suggesting that their were new techniques he also acknowledged their improvements:

"Their technique is good, and in one point (the securing of a more uniform injection) they have improved on the original technique".

He goes on to say:

"There may be some advantage in collecting a number of small successive samples during passage of the blood mixture. These can then be examined at leisure . . . . The total time of injection should be shorter with procedure II (instantaneous injection) than with procedure I (constant infusion) so that collection may be completed before a round of the circulation has been

made by any appreciable part of the salt. Henriques has discussed this point in his excellent paper".

Regarding his use of sodium chloride solution as an indicator in his original paper on cardiac output in 1897, he comments that serum could well be used as an indicator, as he had found the electrical resistance of serum to be two to five times less than that of blood. In his later paper (1921 a) he suggests that perhaps vital red could be used by intravenous injection, and rapid successive samples collected from arterial puncture in man. He concludes his article, however, quite openly advocating the Fick method for determination of the cardiac output in man.

Following Stewart's suggestion of the possible application of indicator-dilution techniques in man, Koch became the first, in 1922, to do so using fluorescin, for circulation times only however. In 1924, Romm described a modification of Stewart's original technique, using a capillary electrometer instead of the telephone system, to record the changes in arterial conductivity, again in the study of circulation times only. He was followed in 1926 by Gross and Mittermaier who pioneered continuous recording of arterial indicator concentration using a string galvanometer, which recorded conductivity changes in the carotid arteries of rabbits during constant rate infusion of sodium chloride into their jugular veins. These workers were also the first to use dye, now that the visual colorimeter had become available.

1927 saw the first use of a radioactive indicator, sodium chloride tagged with radium C, by Blumgart and Yens, and Blumgart and Weiss. They too were only studying circulation times. They made a further advance however, in that they detected the indicator by external scanning, thus presaging future radiocardiographic methods.

The most important advance in the development of indicator-dilution techniques, since Stewart's original description thirty years before, was made in 1928 by Hamilton's group at Louisville (Hamilton, Moore, Kinsman and Spurling, 1928 a). They applied the method for the first time in man, injecting phenoltetraiodophthalein in physiological saline into an arm vein, and sampling from a radial artery needle at rapid intervals into a series of tubes attached to a modified Harvard kymograph. The dye concentrations of these blood samples were estimated in a microcolorimeter. They were able to detect the onset of recirculation in the curves, and appreciated its significance; but on a linear plot they made an arbitrary estimation of curve area, while suggesting that further observations might yield a method of distinguishing the primarycurve, uncontaminated by recirculated indicator. The solution was soon forthcoming. In 1929, came their description of the semilogarithmic replot and extrapolation to define the primary curve (Kinsman, Moore and Hamilton, 1929). Work on waterfilled glass models, with and without recirculation revealed that, where no recirculation was allowed, the downslope of the curve gradually approached the baseline, but would do so only at infinity. This suggested the use of a logarithmic scale, and they therefore plotted their curves on a semilogarithmic scale, whose abcissae (time) were linear, and whose ordinates (concentration) were logarithmic. They discussed the method of calculation of the average concentration of dye flowing during the primary curve, realising that for strict mathematical accuracy the curve should be integrated. To overcome this complicated procedure they compromised by averaging readings taken at secondly intervals. In the same paper, by means of their models, they demonstrated that sampling

from arteries of varying calibre did not affect the validity of the method. In the same journal they published a comparison of cardiac outputs in dogs by the indicator-dilution method and the then widely-accepted Fick method, showing insignificant non-systematic differences in results (Moore, Kinsman, Hamilton and Spurling, 1929). These two papers did more to establish the soundness of the indicator-dilution method than any work published before or since on the subject.

Further excellent work from the same group next appeared on the use of the technique during heart-lung perfusions of dogs (Hamilton, Moore, Kinsman and Spurling, 1930; 1932). They prevented recirculation, and were able to demonstrate that the primary curve did obey the straight semilogarithmic replot, as they had postulated on the basis of their model work. They were now using vital red, a dye suggested by Stewart in 1921, as they discovered that phenoltetraiodophthalein in saline diffused out of the lung capillaries in varying amounts, when checked by simultaneous calculations performed in the heart-lung preparations with both dyes. Direct volumetric checks on the blood flow perfusing the preparations showed that brilliant vital red gave accurate flows (differences averaging -0.7 per cent.), whereas the phenoltetraiodophthalein gave flows averaging 23.9 per cent. too great. Successive cardiac output determinations in man using the same two indicators showed a systematic difference of only 11.2 per cent. The reason for the greater error in the heart-lung preparation work was due to artificially increased pulmonary capillary permeability. It is obvious that the use of phenoltetraiodophthalein in their comparison with the Fick method in dogs (Moore et al., 1929) must

have introduced errors due to sequestration of indicator in the lungs, but they felt that these must have been hidden by the inaccuracies of both the injection and the Fick methods in their hands.

Stewart (1897; 1921 b), and Blumgart and Weiss (1927) both accepted that the fastest circulation time did not differ materially from the mean transit time, because, in flowing through a complicated capillary pathway, a particle of dye is passed in and out of the axial stream so many times that its average velocity would in the end be no different from that of all other particles in the same stream. They overlooked however, the fact that some pathways are longer and more tortuous than other, and hence particles take different times to traverse them. Hamilton and co-workers set about correcting this misconception from their observations in models. They introduced the use of the mean transit time in the calculation of central blood volume, but initially arrived at mean transit time by an incorrect method using the ordinate dividing the curve into two equal parts (Hamilton, Moore, Kinsman and Spurling, 1928 b). This false deduction was caused by their curves being very nearly symmetrical; subsequently however, they corrected this when working with the asymmetrical curves of their heart-lung preparation, and expounded the presently accepted method of arriving at mean transit time, and the vague anatomical boundaries of the central blood volume with its contemporaneous arterial and venous channels (Hamilton et al., 1932).

The instantaneous injection method was now firmly established, and Hamilton's team subsequently went on to employ it in a variety of studies of cardiac output, central blood volume and circulation times, as

influenced by drugs, posture, anaemia, haemorrhagic shock, and various cardiovascular disorders (Hamilton, Moore, Kinsman and Spurling, 1929; 1932; Hamilton, Moore and Kinsman, 1931; Hamilton, 1932).

About the same time, Forssmann (1929) using an ordinary varnish catheter, catheterised his own heart several times,while the following year Klein (1930) drew mixed venous blood from such a catheter and calculated the cardiac output by the Fick principle. Cardiac catheterisation was applied during the next decade to the visualisation of radio-opaque media, injected into the cardiac chambers, and it was not until 1941, by which time a non-wettable plastic catheter had been introduced, that Cournand and co-workers started their invaluable studies on pressures and flow in the central circulation.

At the time that Hamilton's group were perfecting their indicator-dilution technique, the physiology world was very much dictated to by the figures of Grollman (1932) for cardiac output, determined by the acetylene method. Hamilton, Spradlin and Saam (1932) however, criticised the method freely, on the basis of what their method had taught them concerning the rapidity of recirculation, which Grollman had claimed to be between twenty-five and thirty seconds. But it was not until 1949 that the error of the acetylene method was finally accepted, with the publication of the comparison of the acetylene and Fick methods (Werkö, Bersens and Lagerlöf, 1949).

Hamilton's group had demonstrated the validity of the instantaneous injection technique, but it was Holt (1944) who further explored the constant-infusion method. He too was the first to use Evans blue, which was to hold its own for the next twelve years as the most widely-used indicator.

Up until then its use had been confined to the measurement of blood volume, and its properties were well-understood (Gregerson and Gibson, 1937; Rawson, 1943). Holt also started the controversy which continued for many years in the medical press regarding the optimum injection and sampling sites, and the uncertainty of achieving a measurable plateau concentration during constant infusion. Holt injected initially into the right atrium, using the injection device of Wiggers (1944), and sampled from the femoral artery in dogs, claiming plateaux lasting from four to six seconds. Subsequently, he sampled from the omohyoid or carotid artery, realising the injection and sampling sites should be as close together as possible (Holt, Rashkind, Bernstein and Greisen, 1946). The method, its modifications, advantages and disadvantages will be discussed when comparing the relative merits of the constant-rate and instantaneous injection methods.

In 1948, the instantaneous injection technique was widely acclaimed by the scientific world when the Hamilton and Cournand groups published their joint dye-Fick comparison (Hamilton, Riley, Attyah, Cournand, Fowell, Himmelstein, Noble, Remington, Richards, Wheeler and Witham, 1948), followed in the next year by the comparison of Werkö, Lagerlöf, Buch, Wehle and Holmgren (1949), both series demonstrating conclusively the accuracy of the indicator-dilution method.

From this point on the proportion of indicator-dilution studies in the literature increased enormously, especially with the nearly simultaneous description by Knutson, Taylor, Ellis and Wood (1950) from the Mayo Clinic, and Friedlich, Heimbecker and Bing (1950) from Baltimore of instruments which could record continuously the concentration of dye in the

blood, thus overcoming the great inconvenience and inaccuracies of intermittent sampling; which had until that time made the Fick the easier method. Friedlich's instrument was modified by Gilford, Gregg, Shadle, Ferguson and Marzetta,(1953), and Milnor, Talbot, McKeever, Marye and Newman (1953), while the Mayo instrument remained essentially unchanged, having the added advantage of being suitable as an earpiece cuvette, thus overcoming the need for arterial puncture (Beard, Nicholson and Wood, 1950; 1951). Subsequent work was to show however, that the earpiece was difficult to calibrate (Beard and Wood, 1951; Warner and Wood, 1953), not as sensitive an instrument, and had a poor dynamic response (Swan and Helmholz, 1957), a characteristic of which workers were becoming increasingly aware in striving for greater accuracy.

Two major problems remained: the need for patients to breathe one hundred per cent. oxygen to achieve absolute stability of recording when using Evans blue, and the limitation on the number of estimations which could be done per patient, due to the cosmetic effect of overdosage with Evans blue (Connolly and Wood, 1954; Taylor and Thorp, 1959). In 1957, the Mayo group introduced indocyanine green (Fox, Brooker, Heseltine, Essex and Wood, 1957), a dye which was to overcome both these problems, and further increase the scope of the diagnostic applications of indicator-dilution techniques in the investigation of congenital heart lesions with shunts, work which was being performed almost entirely by Wood's group at the Mayo Clinic.

Continuous Recording Techniques

This is an appropriate stage to discuss the advances which brought about relief from the tedious method of multiple sample analysis using dye as an indicator. The method of intermittent sampling at secondly intervals resulted in twenty to forty individual samples per average cardiac output. These had to be centrifuged, pipetted and diluted, in some cases with alcohol to eliminate fatty turbidity and adventitious protein-bound colour (Dow and Pickering, 1950). Merriman, Wyant, Bray and McGeachy, (1953), estimated that this process with spectrophotometry of each specimen took two technicians an average of two and a half hours per cardiac output. Moreover, this process of sampling entailed a blood loss of up to 40 ml. per estimation of cardiac output, thus limiting the number of estimations possible in any one individual, before causing physiological changes due to hypovolaemia. Furthermore, this technique introduced errors in indicator-dilution studies. Events on the curve such as appearance time, peak concentration, etc., could be measured with an accuracy no greater than to the nearest second, which interfered with the use of empirical formulae for the area of the curve, as they depend on accurate measurement of suchparameters (Dow, 1956; Thorburn, 1961). For the same reason, short plateau times in constant infusion dye traces were easily missed with intermittent sampling methods (Howard, Hamilton and Dow, 1953; Peterson, Helrich, Greene, Taylor and Choquette, 1954; Shepherd, Bowers and Wood, 1955). Reasons for a method of continuous recording were therefore amply justified, especially where blood loss should be avoided.

Four types of detecting device can be used for continuous recording of changes in the properties of blood caused by indicator substance:

i) A detector inserted directly into the bloodstream will record changes in conductivity, temperature or optical density.

ii) Another type of detector straddles or encloses a segment of unopened artery, monitoring changes in conductivity or optical density of the bloodstream.

iii) A further method entails sucking blood through a catheter, via a sensing device, after which the blood is discarded, mixed and analysed, or returned to the bloodstream. This can be used for optical density or radioactivity studies.

iv) This typedetects the changing intensity of radio-activity through the intact skin, or measures changes of opacity due to the presence of indicator substance, through an intact transilluminated part, like the pinna of the ear.

Gross and Mittermaier (1926) were the first to pioneer continuous recording of changes in arterial concentration, by the use of a string galvanometer to record changes in conductivity of the blood during constant infusions of salt. White (1947) improved on this by continuous recording of changes in conductivity with micro-electrodes inserted into an artery, but, like previous workers with saline, encountered difficulty with loss of indicator by diffusion in the lungs.

Continuous optical recording and its subsequent application to the measurement of oxygen saturation, and eventually to dye concentration in the 1950's, owed a great deal to the earlier workers on oximetry. In 1934, Kramer described the first instrument for continuous measurement of oxygen saturation in intact vessels (Kramer, 1934 a; 1934 b), and in the same year Matthes described a similar device for use with opened vessels (Matthes, 1934). It was adapted for use with intact tissues such as the ear lobe and pinna, and applied to studies in man (Matthes, 1935). It simultaneously measured the absorption at two different wavelengths, red and green, and was able to distinguish between the effects of changes in haemoglobin concentration and in oxygen saturation (Matthes, 1934). This was the first application of oximetry to continuous recording of indicator concentration in flowing whole blood, measured by the dilution of the blood, as reflected in alterations of the red and green light transmission produced by rapid injection of saline. As was so often the case in the advances of indicator-dilution methodology, the purpose was the measurement of circulation times.

In 1939, Matthes and Gross introduced the use of infrared light, rather than green, as the second wavelength (Matthes and Gross, 1939 a,b,c), a considerable advance in view of the greater light transmission by haemoglobin in the infrared. In the same year, Matthes and Schleicher (1939) used the oximeter to detect methylene blue, again however, for the measurement of circulation times.

In 1942, Goldie and Millikan were able to combine red-infrared measurements to obtain direct oxygen saturation readings, and so popularised the instrument (Goldie, 1942; Millikan, 1942). Wood and Geraci (1949) subsequently developed the basic oximeter to obtain absolute oxygen saturation values directly, without setting the instrument at any known saturation at the beginning of each measurement.

In 1950, workers at the Mayo Clinic described a method of continuous recording of methylene blue and Evans blue concentration in blood, based on their previous work on oximetry (Knutson et al., 1950; Nicholson and Wood, 1950), while a Baltimore group simultaneously reported a photometer for the recording of Evans blue indicator-dilution curves (Friedlich et al., 1950). The latter instrument employed a multiplier phototube for the photosensitive element, a cuvette with plane parallel faces, and high efficiency interference filters to isolate a narrow spectral band centred at 628 mμ, while blood was sucked through the instrument by constant-flow dropping of mercury. It was influenced by fluctuations of oxygen saturation, had a low sensitivity to Evans blue, requiring 20 - 40 milligrams per injection, and use of the photo-multiplier tube for a long period caused fatigue of the cell with instability of the tube's output, so that with direct recording the sensitivity was unstable. In addition, it needed long connecting tubes between the intra-arterial catheter or needle and the cuvette, causing serious distortion of the dye curves.

This instrument was subsequently modified, by adaptation of another property of the multiplier phototube using a "feed-back" system (Gilford et al., 1953; Milnor et al., 1953; Shadle, Ferguson, Gregg and Gilford, 1953).

The anode current was not measured, but used to decrease the conductance of a tube in series with the dynode power supply, tending thus to keep the output constant, irrespective of variations in illumination. The recorded dynode voltage was then proportional to the logarithm of the light intensity, and was strictly proportional over a very wide range interposed between light and phototube. The instrument therefore gave a stable output, and its sensitivity was not affected over a wide range of light intensities.

The division of the apparatus into two main units, a power amplifier unit and a photo-electric pickup unit, made it possible to place the photo-electric unit containing the cuvette close to the patient, thus reducing the connecting tubing, and improving the dynamic response characteristics. The apparatus was nevertheless extremely bulky and not conveniently used on the ear, or on an unopened artery, (Dow, 1956). It allowed greater amplification, and was thus more sensitive to Evans blue, but along with this it had the obvious disadvantage of greater sensitivity to oxygen saturation interference, and there was no compensation for non-specific optical density changes or changes in haemoglobin content, as in the differential photometer.

Falholt and Kaiser (1955) further modified the instrument to try to overcome these difficulties, using two wavelengths of light. Between 545 and 570 mμ oxygen saturation changes give variations in density of the same direction as those from 585 - 650 mμ. They therefore used green light with maximum irradiation at 560 mμ, and a band width of 20 mμ in counterphase with red light to correct for changes in oxygen saturation.

Since the density of whole blood is so much greater in the green part of the spectrum than in the red, they employed a thinner layer of blood for the green light, than the layer of blood absorbing the same percentage of red light. A correction for changes in oxygen saturation could then be obtained by constructing the cuvette so that its depth in the red area resulted in an equal percentage of absorption of light, as that of a given depth in the green area. The depths of the cuvette in the area over the red and green light sources respectively, which theoretically would give the best correction for changes in oxygen saturation, were calculated from the extinction curves of whole blood, the irradiation curves of the lamps, the transmission curves of the filters used, and the sensitivity curve of the photo-multiplier tube, taking into account also the optimum hydraulic factors. Ideally, of course, they would have preferred a compensatory band in the infrared region, but no suitable photo-multiplier tube with sensitivity in this region of the spectrum was available.

Despite these ingenious and painstaking modifications, the oxygen saturation problem was not solved, for although their modification should theoretically have proved satisfactory, they reduced the deflection caused by changes in oxygen saturation to only 23 per cent. of their expected values, a change of one per cent. oxygen saturation causing a deflection equivalent to that caused by a concentration of 0.5 mg./l. of Evans blue in blood (Falholt, 1958).

The Mayo instrument, on the other hand, was a modification of their oximeter, which they had so painstakingly developed. The output of barrier-layer photocells was led, without amplification, through low

resistance recording and balancing circuits. The apparatus measured the difference between the transmission through the blood of red and infrared light. Since Evans blue absorbs light at a wavelength of 625 mμ quite strongly, as does reduced haemoglobin, the oximetric deflection was proportional to the amount of dye, just as it was to the amount of reduced haemoglobin. Therefore, it would produce a deflection that was proportional to the amount of dye in the lightpath, provided that the haemoglobin was itself completely oxygenated. The infrared sensitivity of the photocells permitted a degree of compensation for the concentration and behaviour of red blood cells, impossible with multiplier phototubes. Unfortunately, however, the calibration was not absolutely linear.

The Mayo Clinic validated the accuracy of their instrument in several publications (Nicholson and Wood, 1950; 1951; Nicholson, Burchell and Wood, 1951), and demonstrated the agreement between cardiac outputs performed simultaneously with cuvette and ear oximeter (Beard et al., 1950; 1951; Beard and Wood, 1951). Ring and co-workers further checked the Mayo instrument by simultaneous multiple sampling during the dye curve, and demonstrated that the cuvette curve did not differ from that constructed from the samples (Ring, Oppenheimer, Baier, Sokalchuk, Bell, Ichtiarowa, Ellis, Lynch and Shapiro, 1951; Ring, Oppenheimer, Sokalchuk and Baier, 1951).

Following the above major break-through in instrumentation, advances came mainly in the field of improvements in the dynamic response characteristics of the systems used. These, and the advantages and accompanying complications, which came with the advent of indocyanine green in 1957, will be discussed later in a subsequent section.

Methods of Indicator-Dilution using Indicators other than Dyes

Although the use of indicator dyes has done most to establish the indicator-dilution technique, various other indicators have been tried, including chemical, electrical, optical, thermal and radioactive. Their original development was inspired mainly by the search for reliable methods of continuous recording of whatever property of the blood had been changed by the indicator concerned. To examine their development in detail would be outside the scope of this thesis. Their place in the spectrum of indicator-dilution techniques will, however, be discussed briefly, partly because their development has contributed to that of the more conventional dye techniques, and also because, at a later stage, the use of indocyanine green in this experimental work, in preference to the other indicator methods, will have to be justified.

Radioactive Indicators: Radioactive indicators were first used by Blumgart and Yens in 1927 for the study of circulation times only. Nylin and his various collaborators used red blood cells steeped in radioactive phosphate in a series of studies measuring cardiac output using intermittent sampling (Nylin and Malm, 1944; Nylin, 1945; Nylin and Hedlund, 1949; Nylin and Celander, 1950), and Dow, Hahn and Hamilton (1946) performed similar work using red cells tagged with radioactive iron.

The first use of praecordial counting with intravenous injection of Na^{24} was reported by Printzmetal and co-workers (Printzmetal, Corday, Bergman, Schwartz and Spritzler, 1948; Printzmetal, Corday, Spritzler and Fleig, 1949), and Waser and Humzinger (1948) independently.

They did not initially try to measure flow in absolute units by this method.

Shortly afterwards, MacIntyre and Pritchard and their associates were able to produce the first successful quantitative recordings of the dilution of injected I^{131} albumin, using a scintillation counter-computer detecting assembly (MacIntyre, Pritchard, Echstein and Friedell, 1951; MacIntyre, Storeasli, Krieger, Pritchard and Friedell, 1952; Pritchard, MacIntyre, Schmidt, Brofman and Moore, 1952). Their comparison with rotameter readings in dogs were clumsy, and required estimation of coronary blood flow, while their comparison with the Fick in patients showed a large scatter, with a systematic error of +5 per cent. They had, however, kept pace with the development of other indicator methods, as it was about the same time that the first continuous recording techniques became available for dyes.

Shortly after, Lawson, Cantrell, Shaw, Blackburn and Adams (1952) recorded dilution curves using P^{32} - soaked red cells by a slightly different method, using a Geiger-Mueller tube, and Omhori, Sasamoto and Hosono (1952) reported a comparison of P^{32}, Evans blue and Fick methods using intermittent sampling. Conn (1955) went on to use a modified counter with K^{42}, which he compared with Fick results in dogs. The question of K^{42} loss in the lungs in pulmonary oedema was raised and dismissed. Unfortunately the argument was weakened by their interpreting loss of indicator as causing an underestimation of cardiac output, whereas in fact it would cause an overestimation. Conn subsequently used his K^{42} technique in assessing mitral valve disease flows

(Conn, Heiman, Blakemore, Kuo and Langford, 1957; Conn, Heiman, Wood, Jumbala and Blakemore, 1957). MacCanon and Horvath (1954), and Crane, Sears, Hackney, Holloway and Collier (1958) checked radioactive methods against the Fick using I^{131} albumin, and obtained similar correlations. Crane and colleagues (Crane, Holloway, Adams and Woodward, 1959; Crane, Holloway, Selvester and Crawford, 1960) also compared Cr^{51}- labelled red cells, I^{131} albumin, I^{131} diodrast and I^{131} rose bengal in studies of their suitability for the determination of cardiac output, and concluded that all but I^{131} rose bengal gave similar values for cardiac output and central blood volume, I^{131} rose bengal being unreliable because of staining of, and loss onto, the tubing conveying the blood to the well counter. I^{131} diodrast had the added advantage of relatively more rapid excretion than the other indicators, making more frequent measurements feasible.

The first quantitative work on the radiocardiogram, introduced by Printzmetal et al. (1948; 1949), and Waser and Hunzinger (1949), was performed by Shipley, Clark, Liebowitz and Krohmer (1953) using I^{131} albumin. Their values for cardiac output were very high, probably due to the inordinately high equilibrium value occurring with the use of a wide angle counter. Shortly after, Huff and colleagues (Huff, Feller and Bogardus, 1954; Huff, Feller, Judd and Bogardus, 1955) followed this, using more extensive collimation of the detector head, and positioning the detector over the base of the heart. They obtained a good correlation with the Fick method in dogs and man, but needed relatively large doses of I^{131} albumin, thus limiting their total number

of estimations of cardiac output to four per patient, to avoid radiation hazards. Several further studies reported favourable comparisons with the Fick or the direct-sampling dilution method (Pritchard, MacIntyre and Moir, 1955; 1958; Mack, Wells and Pollock, 1957; MacIntyre, Pritchard and Moir, 1958; Schreiner, Lovejoy and Yu, 1958; 1959; Shackman, 1958; Pritchard, MacIntyre, Moir and Gott, 1959; Shapiro and Sharpe, 1959). These various results gave a mean systematic difference of less than 12 per cent. from the value for cardiac output obtained by the standard technique, with a standard deviation varying between $\pm$ 10 and $\pm$ 20 per cent. Two recent reports however, contradict the favourable results obtained by other workers (Carter, Johnsen, Loeffler and Southward, 1959; Gorten and Gunnells, 1960; 1961).

Recent work from Cournand's laboratory gives radiocardiogram studies new interest (Cournand, Donato, Durand, Rochester, Parker, Harvey and Lewis, 1960). They injected Kr^{85}, which is dissipated in the lungs, into the venous circulation, and thus obtained pure right ventricular curves. They then injected I^{131} albumin into the pulmonary artery and obtained pure left ventricular curves. They thus overcame the problem of the extrapolation of the otherwise ill-defined right ventricular downslope in a double curve, obtained by radiocardiography using one radioactive indicator for both ventricles.

Thermal Dilution Techniques: In 1953 Fegler showed that, following intravenous injection of cooled blood or Ringer's solution, he could record temperature-time curves by means of thermocouples placed in either the pulmonary artery or the aorta, and that these curves bore a close

resemblance to dye-dilution curves (Fegler, 1953; 1954). He then attempted to measure the flow of water through a model circulation by thermodilution with such success when the model was contained within an airjacket, that he was encouraged to try the method for the measurement of cardiac output, since the pulmonary circulation might be expected to possess similar properties of thermal insulation. He obtained very close agreement between thermodilution and simultaneous dye-dilution and direct Fick measurements of cardiac output in a small series of experiments on dogs.

His remarkable work was received with incredulity, especially by Dow, who was frankly sceptical in his revue (Dow, 1956). Three subsequent papers however, compared thermodilution with dye-dilution and the direct Fick method, and reported close agreement of all three techniques, thermodilution being without systematic deviation with respect to the other two (Fegler, 1957; Goodyer, Huvos, Eckhardt and Ostberg, 1959; Klussman, Koenig and Lütcke, 1959).

Fronek and Ganz (1960) further modified the principle for measuring flow in vessels such as the carotid artery and jugular vein. They injected cooled saline or dextrose in an upstream jet with sufficient velocity to cause adequate mixing over a full cross section of the vessel. They then registered temperature change only a few millimetres downstream by means of a thermistor attached to the catheter. This method largely overcame the objection of heat exchange between the point of injection and the temperature-sensing device. It has definite shortcomings in the measurement of cardiac output however, as pointed out by Hosie (1962),

in that, with use in the pulmonary artery, it requires repeated curves to obtain a true average cardiac output in view of the rapidly fluctuating pulmonary artery flow with respiration.

Another recent advance in thermistor techniques has been the use of injectate at a temperature nearer that of body temperature, when measuring cardiac output with injection and sampling sites on opposite sides of the pulmonary bed (Evonuk, Imig, Greenfield and Eckstein, 1961). This lowers the blood-tissue temperature gradient and so minimizes heat loss to the tissues. A questionable disadvantage of the technique, however, has been that, unlike the dye method, central blood volume estimations are impossible, where cardiac output is measured with both injector and thermistor mounted on the same catheter in the pulmonary artery, as described by Froněk and Ganz (1960). Where the indicator is injected on the venous side and sampled from the arterial side of the circulation, the time distortion of the curve by the exchange and re-exchange of heat with the tissues makes derivation of mean transit time, and so central blood volume, erroneous.

Conductivity Methods: Sporadic interest has been shown in the use of indicators which change the blood conductivity ever since Stewart first used sodium chloride solution in his original work in 1897, despite the fact that errors were caused by indicator loss in the lungs. Gross and Mittermaier substituted a string galvanometer to achieve continuous recordings of the changing conductivity of blood after saline injection in 1926. Wiggers (1944) improved the sampling method, and used a more accurate method of electro-titration of the control and unknown

blood-saline sample. White (1947) made a considerable advance with the insertion of microelectrodes into the artery for continuous recording of conductivity changes. He found difficulty in avoiding interference with the record by capacity or resistance changes due to movements of the arterial wall with respect to the electrodes, and in ensuring that the salt-blood mixture gained free access to the electrode surfaces, especially in the small vessels of the dogs which he studied. Gray and Paton (1949) used the method in the study of circulation times in cats, and Ingraham (1949) modified White's method by improvements in the electronics to allow a smaller injectate, thus reducing indicator loss in the lungs.

In 1956, Holt employed saline as an indicator in studies on the residual volume of the left ventricle in dogs. He pointed out the many pitfalls in calibration with this method which allow many sources of error. The following year, Booth, Ryan and Goodwin (1957) used the method in the detection of cardiac shunts. In Holt's work diffusion difficulties did not arise, and in the studies of Booth's group they were not significant, as accurate quantitative estimates were not attempted.

Goodwin and Sapirstein (1957) used plasma as an injectate to overcome the loss of indicator in the lungs. They had to calculate a "form-factor" which described the shape and orientation of the red blood cells with respect to the electrodes for every new conductivity cell and for every withdrawal rate. A considerable quantity of blood must have been necessary to obtain the large volume of plasma for injection (5-10 ml./l./min.

of expected cardiac output) necessary to minimise the artefacts caused by the orientation effects of the cardiac cycle on the red blood cells, and the slower changes in resistance associated with respiration.

Hershgold, Steiner and Sapirstein (1960) have improved the method by electronic damping of the detecting circuits, allowing greater amplification of the signal, without increasing the variability of the baseline occurring during each cardiac cycle. They used a solution iso-osmolar and iso-conductive with plasma, instead of autogenous plasma, but still required about seven millilitres per injection to obtain satisfactory curves in a dog with a cardiac output of four litres per minute.

Clark (1960) has recently described the use of sodium ascorbate, one of a group of chemical reducing agents, as an indicator, with intravascular platinum electrode recording of indicator-dilution curves by an amperometric technique. The method is well-suited to diagnostic cardiac catheterization procedures because of its simplicity and the avoidance of costly equipment (Frommer, Pfaff and Braunwald, 1961; Nixon, Hay, Hepburn, Snow and Addyman, 1963).

Calibration for quantitative estimation of cardiac output has not yet been described and would seem to present considerable difficulties. In common with other conductivity methods, indicator loss occurs to some extent in the lungs, and artefacts may result from movement of the subject (Frommer et al., 1961).

Haemodilution Methods: This method is mentioned finally for completeness, although it has not been a serious contender with the other methods,

despite the simplicity of influencing the optical density of blood by injecting a transparent substance and so reducing the optical density of haemoglobin by simple dilution.

In 1934, Matthes applied his oximeter to the first recordings of indicator concentration, measured by the dilution of blood produced by rapid injection of saline, and as reflected in alterations in red and green light transmission. Kramer and Sarre (1935) described similar experiments the following year, but both studies were directed to the measurement of circulation times only. Workers from Göttingen showed that right heart injections of Ringer's solution gave transient dilutions of the blood, which were recordable as increases in light transmission through the exposed carotid artery, placed in a narrow trough between a water-cooled lamp and a barrier-layer photocell (Lochner and Schoedel, 1950; 1952 a,b; Heller, Lochner and Schoedel, 1951; Heller, Kaiser, Lochner and Schoedel, 1953). These initial efforts did not measure cardiac output in absolute units, but, by keeping the injection dose constant, the area of the dilution curve became an index of whether the cardiac output was rising or falling in each animal. Heller et al. (1951) also showed the inconstant errors of using Ringer's solution, caused by its diffusion across the lungs, and thus switched to the use of plasma, which could be relied upon to remain intravascularly in its passage through the lesser circulation. Their "cardiac photo-sound", introduced into the right ventricle, was also used for dilution curves, but these were again not calibrated, although this device approached the ideal of recording at the catheter tip, since the lamp, photocell and a slot for the blood were actually inside the right ventricle (Heller et al., 1953).

Lochner and dal Ri (1957) have used nondiffusable dextran as an indicator for this technique. Unfortunately however, very large quantities of indicator, whether saline, plasma or dextran, have to be injected in order to bring about the equivalent change in optical density produced by a few milligrams of Evans blue at the appropriate wavelength, and calibration in absolute quantitative terms remains elusive.

Interest in haemodilution methods has recently been revived by a discussion of the errors which may be caused in densitometer use by non-specific substances such as saline, injected in company with indocyanine green, and their correction by dichromatic densitometry (Sinclair, Sutterer, Fox and Wood, 1960; 1961). What constitutes a method of measuring cardiac output has therefore become a potential complication of monochromatic densitometric techniques.

THE THEORY OF INDICATOR-DILUTION METHODS FOR MEASURING FLOW

Figure 1 shows a dye curve recorded by the method used in the present investigation in a normal subject, with the injection catheter tip in the right atrium and the sampling catheter tip in the aortic root. The dye concentration will be seen to rise to a peak, and then fall away until an obvious second curve occurs due to the bulk of the dye returning to the heart, greatly diluted. Unlike dye curves commonly illustrated in studies by earlier workers, it is apparent that the recirculation "hump" occurs very much later, and not on the downslope of the curve. This has been achieved in the present system by centrally placed injection and sampling catheters. However, the ready separation of the recirculation "hump" from the primary curve has not entirely eliminated the need for separation of recirculated dye contributing to the curve area, as, hidden within the apparently smooth downslope, is the dye which has recirculated more rapidly through vascular beds with short pathways such as the coronary and thyroid circulation. Two problems will therefore be considered; is the dye curve an adequate method of measuring flow, and how can the problem of premature recirculation be overcome to obtain the area of the primary curve alone?

Indicator-Dilution Methods as a Means of Calculating Flow

The technique of indicator-dilution for measuring flow undoubtedly stemmed from Stewart's earlier work on circulation time. The method is based on the principle that, if a marking substance be injected into a flowing liquid, the degree of dilution after adequate mixing is a measure of the volume of flow. In a closed system the measurement must be made

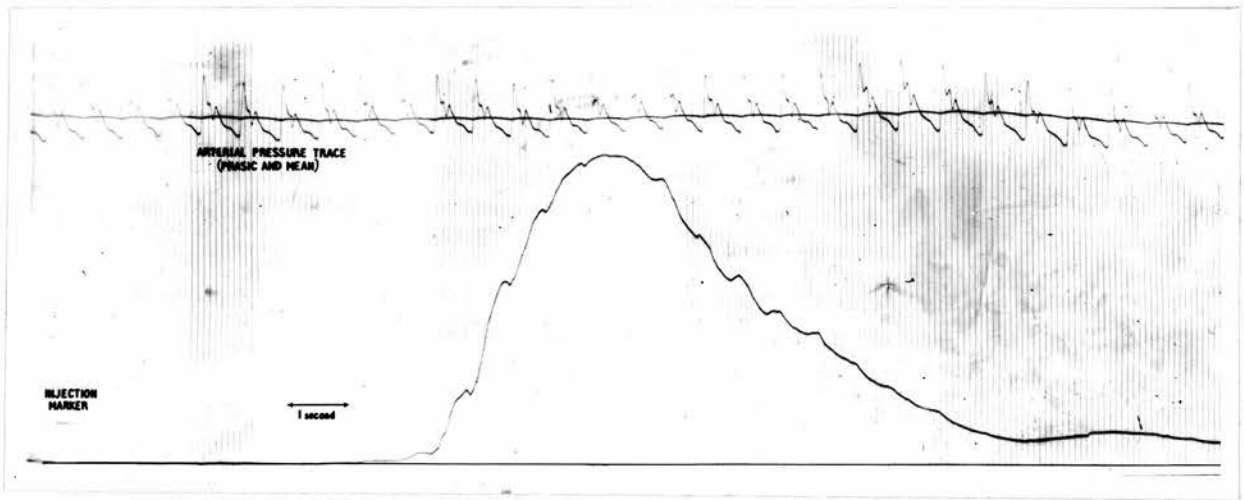

Figure 1: Normal dye-dilution curve for cardiac output

during the first circulation of the injected substance. The method has come to be used in two different ways, instantaneous injection and constant infusion, the former being preferred in the present study, for reasons which will be discussed in a later section.

The theoretical principles behind the validity of this method as a means of measuring flow have been presented by numerous workers (Kinsman et al., 1929; Stephenson, 1948; Cyvin, 1949; Nylin and Celander, 1950; Meier and Zierler, 1954; Sheppard, 1954; Burger, van der Feer and Douma, 1956; van der Feer, 1958; Zierler, 1958; 1962 a,b). The following assumptions are implicit in the calculation of flow and volume by the method:

i) The distribution of traversal times for fluid particles entering the system must not change with time ("stationarity of flow"). This is common to all indirect methods of measuring cardiac output, and implies that the subject must be in a steady state during the inscription of the indicator-dilution curve. This premise is violated in the pulsatile vascular system. If, however, phasic, not necessarily regular, alterations in the distribution of transit times fluctuate rapidly about some central value, and if the periods of the phases are brief compared to the time required for the evolution of the instantaneous injection indicator concentration-time curve, then the violation of "stationarity" is unimportant.

ii) The distribution of traversal times for the indicator must be representative for the whole fluid. This is not a source of error in the method used in this study where whole blood is sampled through a densitometer.

iii) There must be no loss of indicator material or sequestration between injection and sampling sites. Loss in transit through the pulmonary circulation was the problem originally encountered by Hamilton's group using phenoltetraiodophthalein as an indicator, but this problem does not arise with the newer indicators such as indocyanine green. Absence of sequestration is necessary only for the measurement of volume, and is not essential in the measurement of flow. Thorburn, Korner and Stephens (1959) have shown that with central injection into the cardiopulmonary circulation, no sequestration occurs, because the pulsation of the elastic walls of the system with each cardiac cycle prevents any delay in clearance of indicator from the heart chambers.

iv) The system must have either a single inflow, or a single outflow, or somewhere within the system there must be a single channel through which all flow must pass, and in which mixing occurs (Rossi, Powers and Dwork, 1953; Zierler, 1962 a). While this creates several problems in measuring peripheral flow (Andres, Zierler, Anderson,

Stainsby, Cader, Ghrayyib and Lilienthal, 1954), the conditions are fully satisfied in the present study.

v) The semilogarithmic extrapolation must account adequately for the primary curve. Circulation model studies have demonstrated the accuracy of flows estimated by the Hamilton semilogarithmic replot method (Kinsman et al., 1929; Thorburn et al., 1959). Its validation against the Fick method offers further indirect proof of its validity, and the principle of the extrapolation will be discussed later in this section.

In the case of constant rate injection of indicator at I mg./min. through a system of fixed volume in which flow is assumed to be constant at Q l./min. without recirculation, eventually the concentration of the indicator in the system will reach a plateau concentration, and the rate of indicator leaving will come to be equal to the rate of its infusion into the circulation. Its rate of leaving the system is a product of outflow concentration Cmax. in mg./l., and the unknown flow, Q:

$I = Q \times Cmax.$, whence $Q = \frac{I}{Cmax.}$

Similarly, in the case of instantaneous injection, indicator injected suddenly into a fluid system appears at the outflow of the system in a concentration which is a curvilinear function of time c(t); the arterial concentration curve being in actual fact simply a frequency distribution of the transit times of all the dye particles from the injection to the detection site (Korner and Shillingford, 1956). The rate at which indicator

leaves the system at any moment, s, is the product of its concentration at that moment, c(s), and the unknown flow in l./min., Q. Eventually the entire mass, I, of injected indicator must leave the system. If all products of c(s) and Q are summed, this sum must equal the amount of injected indicator: $60I = Q \int_0^\infty c(t)\,dt$ from which: $Q = \frac{60I}{\int_0^\infty c(t)\,dt}$.

For strict mathematical accuracy the curve should be integrated, but the mathematical operations of this are so complicated that, for practical purposes, the average of readings taken at finite frequent intervals suffice. Q, the unknown flow, can therefore be measured from the equations given for constant infusion or instantaneous injection.

Hamilton and Remington (1947) and Lewis (1953 a) pointed out that constant rate injection could be considered an integral of instantaneous injection, and that Cmax. must therefore be the integral of the concentration-time curve obtained by instantaneous injection. Since both methods measure the unknown flow, Q, the equations can be combined: $\frac{Ii}{Ic} = \frac{\int_0^\infty c(t)\,dt}{Cmax.}$. If Ii + Ic are so chosen that $\frac{Ii}{Ic} = 1$, then $Cmax. = \int_0^\infty c(t)\,dt$.

The Problem of Recirculation

The above discussion has applied to the situation where recirculation does not complicate the calculation of flow. In vivo, however, recirculation causes the indicator concentration at outflow to be augmented by recirculating indicator before its concentration has returned to zero in instantaneous injection curves, and in the case of constant rate infusion, before the plateau concentration is reached. There are two main ways to handle the problems created by recirculation:

i) One is to extend the treatment of the concentration of indicator as a function of flow and volume to include the case of recirculation.

ii) The other is to treat formally the concentration of indicator during its first circulation as a separate event from the obscured concentration in the presence of recirculation and, by some means, extract from the overall concentration-time curve the concentration-time curve representative of the first circulation alone (Zierler, 1962, b).

The possibility of including the indicator due to recirculation in the fundamental equation requires a redesign of the experimental method, depending on sampling from two sites, or injecting and sampling at two sites (Stephenson, 1948; Meier and Zierler, 1954; Cheesman, Gonzalez-Fernandez and Wood, 1959; Parrish, Hayden, Garrett and Huff, 1959). The methods are complex, both experimentally and analytically, although they have the advantage of making no assumptions about the form of the distribution function. It would be far simpler if the shape of the distribution function were predictable, so that its distortion by recirculating indicator could be eliminated by extrapolation of the distribution function beneath the distortion, according to either the downslope of the function, or by the restriction of the entire distribution function to some formal expression.

The Hamilton Semilogarithmic Replot Method and its Inherent Errors

Hamilton and his group showed that when an indicator was injected suddenly intravenously in the dog, and the concentration of indicator measured with time in arterial blood, the downlimb of the time-concentration curve sooner or later fitted an exponential of the form $C_t = C_{oe} - \frac{Q}{XV} t$ until it was interrupted by an increase in concentration attributed to the first recirculation of indicator. In the above expression, Ct is any arbitrarily selected concentration on the proper part of the downlimb in mg./l. after the elapse of t seconds, Co the original concentration in mg./l., e is the mathematical constant 2.7183, the base of natural logarithms, Q is the flow in l./sec., V is the volume of the system in litres, and X is a factor to correct for non-instantaneous mixing (Hamilton et al., 1928, b). The expression for exponential decay is one in which the rate of fall of concentration at any time is proportional to the concentration at that time. A logarithmic replot of indicator concentration against linear time therefore yields a reasonably straight line until recirculation occurs. When recirculation does occur, the logarithmic downslope is simply extrapolated to very small concentrations (to infinity theoretically). This corrected time-concentration curve is then replotted on linear co-ordinates, and, with recirculation eliminated, the area of the primary curve may be planimetred.

Indicator passing through straight tubes (Rossi et al., 1953), or a tubular arteriovenous network such as the pulmonary vasculature (Howard et al., 1953; Schambye, 1953) does not give an exponential downslope.

This may be due to the fundamental nature of laminar flow in transporting indicator (Rossi et al., 1953), or due to the fact that organs such as the lung and kidney have rapid and slow circulatory channels with differing volume-flow relationships. When glass models with different volume-flow ratios are arranged in parallel, the washout time-concentration curve is not exponential and so cannot be extrapolated (Hamilton et al., 1932). Likewise, the descending curve from lungs perfused through the pulmonary artery and sampled at the pulmonary vein is also not exponential (Howard et al., 1953). Both these curves become exponential when a mixing chamber is introduced in series. The mixing chamber serves to smooth out the irregularities of the curve as it leaves the complex system, and impress upon the downslope the exponential washout form that would be expected from dye leaving a single reservoir. Unless special precautions are taken to ensure instantaneous mixing of dye in a glass chamber model, the volume of the chamber is not indicated by the time-concentration curve obtained from the effluent stream (Hamilton et al., 1932; Newman, Merrell, Genecin, Monge, Milnor and McKeever, 1951). Nevertheless, whether mixing is complete or not, the flow through the chamber can be accurately calculated.

The method introduced by Hamilton's group has proved an extraordinarily useful artifice, although there is no convincing theoretical reason, according to Dow, for the downlimb to assume the form it does, and, as he rightly points out, it does not do so in every case (Dow, 1956).

The importance of the accuracy of the semilogarithmic replot method for excluding recirculating indicator cannot be overstressed. Any doubt

of its validity would lay open to question the results presented in this thesis in which the method was employed.

Sutton, Karnell and Nylin (1950) studied just how soon recirculating indicator contaminated the primary curve, by constant infusion of radioactive phosphorus (P32)-labelled red cells into the pulmonary artery with serial sampling from the right ventricle. Their findings led them to conclude that blood traversing the fastest circuits, such as the coronary bed, could well reappear at the sampling site before the exponential downslope had established itself. They felt that the time on the descending limb,at which there was a definite deviation from the straight semilogarithmic replot of falling concentration, was merely the point at which the proportion of recirculating indicator was increasing as fast as, or more quickly than, the proportion of initially circulating indicator was falling, thus creating a break in the total concentration curve. This was not therefore the time at which recirculation began, but the time at which it became manifest, and that the error tended to hide itself. The greater its amount, and the earlier its occurrence, the more the recirculating indicator tended to contribute to the formation of the descending limb, making its descent more gradual, and thus prolonging the point at which its contribution made the time-concentration curve break its smooth descent.

While agreeing with the significance of their theorizing, as Dow has pointed out, the conclusion drawn from their experimental results are dubious, and recirculation would probably have occurred no sooner than fifteen seconds after injection, roughly double their calculated time.

They present no figures for appearance times in their study, and the hypothetical arterial dilution curve upon which they plot their findings seems quite unrealistically distorted (Dow, 1956).

If recirculation is a late event in the instantaneous injection method, as indeed it is in the present study, dye concentration has returned almost to zero before the effect of recirculation is evident. Under such circumstances the exponential extrapolation method of Hamilton is quite adequate.

Etsten and Li (1954) reported that not all indicator-dilution curves followed an exponential decay, and that in some curves the lower part of the descending limb is deflected inwards. They suggested that this was due to an alteration in cardiac output during the inscription of the curve. Carleton, Abelmann and Levinson (1960) confirmed this impression in an elegant study in man and models by producing acceleration of blood flow during the dilution process. In addition, they obtained similarly distorted curves in models with two sequential chambers with large ratios of residual to total volume in the indicator path. Spontaneous continuously accelerating decay limbs were found far more frequently in patients with mitral or pulmonary stenosis than in normals. This finding they attributed to the large intracardiac residual volumes occurring in these cardiac patients. In their models, calculated flow compared well with volumetrically measured flow, and in man the indicator-dilution results agreed closely with Fick estimations. With artificially induced accelerating curves the model flow values were wrong, falling between basic and accelerated flows, but not giving the true flow, as measured into a receptacle. The effect of an

increase in flow could be predicted. With a stable heartrate, an increasing ejection volume, decreasing residual volume, or a combination, will define a decreasing ratio of residual to total volume. The resultant decay limb will be progressively steeper, and apparent exponential decay will be prevented for a prolonged period of time.

The low incidence of deformed curves in normal subjects suggested that there was little stimulus to acute changes in flow during conditions of right heart catheterization. There is no evidence that patients with mitral or pulmonary stenosis are more liable to acute changes in flow than normal subjects under similar conditions of study. On the other hand, the frequent occurrence of dilatation of the right ventricle in pulmonary stenosis, and of the left atrium in mitral stenosis, suggested that the continuously accelerating decay limbs in the curves from their subjects were analagous to those produced by large ratios of residual to total volume in the model.

In a biological system with two or more chambers in which recirculation occurs, all dilution curves, when precisely analysed, have accelerating decay of indicator concentration persisting through any finite time period. With less stringent criteria, most curves have a segment which closely approximates exponential decay. Errors in flow estimates resulting from extrapolation of such a segment are small, and of little significance. Furthermore, as any of the chambers traversed is enlarged, the normal visually apparent period of accelerating decay will be prolonged, but only in the extreme case may persist beyond the onset of recirculation. On the basis of a family of mathematically derived curves, Carleton et al. (1960)

defined 0.85 as the upper limit of the ratio of residual volume: residual plus ejection volume in sequential chambers, above which the formation of an apparently exponential segment within the usual time limits imposed by recirculation would not occur.

In the present study, the vast majority of downslopes followed an exponential decay before recirculation occurred. Occasionally however, in normals, and patients with valvular heart disease, continuously accelerating decay limbs were observed. Simply joining the replotted points in the absence of an exponential portion did not seem to influence the accuracy of the technique when values were compared with those of the Fick method.

Etsten and Li (1954) employed the formula of geometric series to compute the area under the exponential portion of the downslope. They pointed out the potential errors of the accelerating curve, and implied that the use of the geometric series formula could cope with this problem. While it is an alternative method of estimating the area under the exponential to infinity, it in no way overcomes the problem of calculating area in a curve with a non-exponential downslope, where recirculation may be hidden, and cannot be excluded by the usual extrapolation method with confidence.

With regard to the problem of choosing an extrapolation "by eye" in difficult curves, it should be pointed out that observer error must play a part in the calculation of curve area. The only reference to this error is by Nylin and Hedlund (1958) in which three different observers calculated the cardiac output from three different dilution curves to the nearest 0.1 l./min.; the means for each observer varied thus: 6.4, 7.0, and 6.0 l./min. This was almost certainly due to differences in choice of the

points of extrapolation of the descending limb. Doyle, Wilson, Lepine and Warren (1963) commented on the element of subjectivity which must enter into the construction of the dilution curve, and stressed the importance of some degree of skill being acquired in drawing the curve before consistently satisfactory results were obtained. In their case however, the problem was complicated by having to construct their curves from intermittent sample values of concentration. This problem is overcome in the present study by continuous recording of concentration. Pulsatile flow however, caused a trace which fluctuated with every heartbeat, and the traces were therefore smoothed freehand in every case before concentration values were measured.

The measurement of dye curves involves taking concentration readings at set time intervals (usually once per second) until an arbitrary concentration is reached, usually 0.1 - 1 mg./l., and not to infinity as would be theoretically correct. Hoffman (1960) has examined the possible error which may result from thus underestimating the curve area, and so overestimating flow. In models in which flow and central volume could be varied, he compared the estimated flow by:

i) collecting and measuring the effluent volume,

ii) calculating flow from the curve by taking values to an arbitrary lowest level,

iii) taking values to infinity.

As would be expected, the calculated flows were greater by the routine estimation to an arbitrary low level of concentration, than when measured

directly, the systematic error being +3 per cent., as the curve area is inversely proportional to flow. This error is of a similar order to those of the model experiments performed by Kinsman et al. (1929), who found errors of + 2.4 per cent. with no recirculation, and + 4.8 per cent. with recirculation. When curve areas were calculated to infinity the error was reduced to + 1 per cent. compared with directly measured flow, a considerable improvement in accuracy. Thorburn et al. (1959) estimated a similar error of about 4 per cent. in not plotting to infinity, but to a concentration of 1 mg./l. (approximately 1/40th of peak concentration) in model experiments. Lack of relationship between the magnitude of the flow and any systematic error is easily explained by the geometry of the curves themselves. At high flows the curve changes shape; it becomes "narrower", its duration shorter, and its fall steeper.

The increment in area is smaller by plotting to infinity, but the area under the curve is also smaller, so that the ratio of the increment in area to the total area remains constant. It is not possible to apply a constant correction for any systematic error, because, although it is independent of flow, it varies with changes in the injected indicator amount and the magnitude of the central volume. With other parameters constant, a change in the amount of injectate alters the area of the curve, but not the shape; the increments in area gained by summing the dye concentrations to infinity are therefore constant, but their ratio to the area under the curve changes. Changes in central volume, with other factors kept constant, causes no change in curve area, but the downslope, and thus the increment in area by an infinity plot, changes. In the presence of significant valvular regurgitation the increments in area

will increase, although the total curve area does not alter.

From the above discussion it can be readily appreciated that the calculation of flow from dye curves by the Hamilton semilogarithmic replot method is not only complicated by potential errors, but is a fairly lengthy procedure.

Lilienfeld and Kovach (1956) developed a graphic method to calculate the area beneath the dye curve, the slope of the downstroke, and the mean transit time. Their method is of particular interest in the calculation of curves with a prolonged downslope, where the arithmetic summation of concentrations at each second is very time-consuming, but the semilogarithmic replot of the curve is still necessary.

In some systems of indicator-dilution techniques with peripheral injection and sampling sites, recirculation occurs early, so that the downlimb of the curve is contaminated before an exponential decay has become established. In such cases no correction for recirculation can be made with confidence by the Hamilton extrapolation method, and it is necessary to use one of the other methods which demand measurement of dye concentration at two sites, such as the procedure proposed by Stephenson (1948). Since the problem did not arise in the present study, the validity of this procedure will not be pursued in this discussion. It is however, of interest to mention some of the other ways in which the recirculation problem has been tackled to obtain true blood flow. One of these has been to examine time-concentration curves obtained experimentally and, by curve fitting, derive an empirical expression for the concentration as a function of time or some other useful relation.

Empirical Expressions used in the Calculation of Flow from Indicator-Dilution Curves

The first empirical expression was derived by Allen and Taylor (1924), who treated as triangles their observed time-concentration curves, obtained following sudden injection, when measuring the volume of water conduits in hydraulic engineering. The area of the curve was then obtained in much the same way as by Warner and Wood (1953) who were looking for a rapid way of calculating area. Their approximations were unsatisfactory for biological application due to the large error, and the fact that recirculation obscured the curve, so that the disappearance time could not be estimated with confidence.

Certain workers, however, have suggested that alternative methods may be used, not to increase the accuracy when extrapolation difficulties arise, but rather to avoid the tedious calculations involved in Hamilton's method, at the expense of some accuracy. Nicholson and Wood (1951) suggested plotting the exponential downslope "by eye" on its linear co-ordinates, which involved simply extending the straightest portion of the downslope, and then planimetring the curve to obtain area. However, since the falling limb is seemingly straight, and the disappearance of dye is exponential, it is not possible to tell when the curve departs from an exponential track. If their procedure is adopted, part of the dye curve area prior to recirculation is omitted, and a factor must be provided to correct the planimetre results for area. Both Nicholson and Wood (1951) and Ring et al. (1952) found that considerable correction factors were necessary, while accuracy was sacrificed.

An even greater simplification of calculation was used by Warner and Wood (1953) who treated the dye curve as a triangle, the three apices of which were the starting point of the deflection, the peak concentration point, and the point where a line drawn from the point of peak concentration along the downslope of the curve intersected the base line. The area of this triangle was calculated from the formula $\frac{Cp.Tp.}{2}$, where Tp is the length of the baseline of the triangle, and Cp the length of the perpendicular dropped from the point of peak concentration to the baseline. Their calculated mean concentration was greater than that obtained by the Hamilton method, but the transit time (baseline of the triangle) was smaller. These systematic errors were opposite in direction, thus reducing the overall systematic error in calculated cardiac output. The inherent variability of the estimates for cardiac output increased from 11 per cent., based on the studies of Werko et al. (1949), Bear and Wood (1951) and Beard, Wood and Clagett (1951), to 14 per cent. using this method.

The next line of attack was via the assumption that indicator-dilution curves "committed" themselves as soon as the rising limb and peak concentration has been inscribed. With this in mind, Dow and Hamilton (Dow and Hamilton, 1950; Dow, 1955) examined a large number of indicator-dilution curves obtained by sudden injection into the cardiopulmonary circuit in dogs and in man, acquired from various sources. After testing various combinations of a number of measurable parameters of the curve, the following formula was arrived at, as correlating best with the true

area under the time-concentration curve, corrected for recirculation:

$$\text{Area} = \frac{T_{PC} . C_p}{3 - \frac{(0.9\ T_{PC})}{T_A}}$$

where TPC is the time in seconds after injection when peak concentration occurs, Cp is the concentration in mg./l. at the height of the curve, and TA is the appearance time in seconds. As pointed out Dow himself at a later date (Dow, 1956), the dye curves from patients were all obtained by intermittent sampling techniques, which inevitably make the measurement of the parameters in the formula both difficult and inaccurate. Even in the densitometer traces of the curves obtained from dogs, their pulsatile nature prevented the measurement of the parameters of time to any accuracy greater than the nearest tenth of a second, at the very best. Errors due to such inaccuracies would vary according to the rapidity of circulation and how centrally placed were the catheters (Thorburn, 1961), and cannot therefore be assessed, as Dow worked with data pooled from different laboratories using different techniques. Nevertheless, Dow himself subsequently pointed out that, even allowing for technical inadequacies in the material used, the formula failed to replace the conventional extrapolation method (Dow, 1956). Keys, Hetzel and Wood (1957) found that, in man, Dow's formula systematically underestimated flow as calculated by Hamilton's method.

Wood's group (Hetzel, Ramirez de Arellano and Wood, 1955) employed a similar approach, comparing the area of what they called the forward triangle with the total area of the curve, corrected for recirculation

by extrapolation. The forward triangle incorporated measurements of buildup time in seconds (TB), the time taken from the appearance of the curve to its peak in seconds, the peak concentration, Cp, and K, a proportionality constant derived from the comparison of the forward triangle estimate of area and the area of the curve as determined by the Hamilton extrapolation method: $K = \frac{\frac{1}{2} T_B \cdot Cp}{Area}$

Flow (by forward triangle) $= \frac{2K.\ 60.\ I}{T_B \cdot C_p}$

The ratio of the forward triangle to the total area of the curve, after excluding recirculation by semilogarithmic replot, K, was determined in a number of experiments in man in which indicator traversed the heart and lungs. The ratio varied somewhat according to the site of injection, but in general was surprisingly stable at a value of about 0.35. Hetzel, Swan, Ramirez de Arellano and Wood (1958) subsequently investigated the effect of the injection site on the value of the constant (K) by injections into the pulmonary artery, superior vena cava and brachial vein in normals and patients with valvular defects. They found that K was smaller when injection was peripheral and larger with central injection. At the same time they calculated cardiac output by Dow's formula, using a correction factor of 1.37, and obtained a fairly good agreement with the value for cardiac output calculated by the standard Hamilton semilogarithmic replot method. Their feeling was that, while the forward triangle method was undoubtedly affected by the site of the injection, the differences between central injection sites on the right side of the heart were not of practical significance. Dow's formula on the other hand, which incorporates appearance time, may compensate to some extent for the effect of varying

injection sites on the relation of the initial components of the curve to its whole.

It was therefore gradually becoming apparent that the degree of asymmetry of a dye curve was influenced by a variety of factors, such as changes in flowrate, volume and pathlength, and that these factors profoundly influenced the estimation of flow by these empirical methods. Thorburn (1961) demonstrated this in model experiments, varying one parameter at a time. The Dow method is even more erroneous than the forward triangle method, as it uses peak concentration time instead of buildup time, the former being even more sensitive to variations in curve asymmetry. The ratio of the area estimates of the two methods will vary as the ratio of peak concentration time to buildup time:

$$\frac{\text{Dow area}}{\text{Forward triangle area}} = K \times \frac{T_{PC}}{T_B}$$

Thorburn (1961) found that he could estimate mean circulation time from appearance time (Korner, Uther, Chalmers and Nicks, 1960), and that if a line were drawn through the curve at this point, the area anterior to this line represented approximately 62.5 per cent. of the total curve area, irrespective of the source of the curves or any variation in flow and volume. This method also caused overestimation of flow at low flows, and underestimation at high flows, similar to that with the Dow and forward triangle methods. The reason is that the ratio MCT:AT is very sensitive to variations in the degree of asymmetry of curves. This ratio, as explained, is influenced by a variety of factors, so that, despite obtaining a fraction of the total area which is independent of variations

in symmetry, the estimations of cardiac output are still affected by the use of a time ratio which is also subject to these variations. Thorburn showed that flow and central volume (dependent on injection and sampling sites) accounted for about 40 per cent. of the initial variation in the factor K of the forward triangle method. A considerable amount of the residual error could probably be explained by variations in path length between individuals. The accuracy of these methods could be somewhat improved by deriving individual constants for each injection site.

Although these empirical correlations may be better than nothing when trying to salvage data, they were obtained in a specified set of experiments, in particular only in those in which recirculation was sufficiently late to permit application of the Hamilton semilogarithmic replot. The necessity for their use arises in quite another set of circumstances in which recirculation is an early event. There is no evidence whether the correlations from the first set of experiments can be applied to the second. Furthermore, none of the correlations applies to experiments in which injection or sampling, or both, are from other parts of the cardiopulmonary circuit, or in which other vascular beds are studied. Finally, these empirical formulae yield approximations only of curve area. They can therefore be used only for estimates of flow, and volume cannot be derived from them (Zierler, 1962, b).

Mathematical Models used to Predict the Form of the Distribution Function

Assuming that certain laws govern the distribution function of transit times, another approach is to predict the distribution function from these laws, and test the observed time-concentration curve for

correlation with the theoretical prediction. As pointed out by Zierler (1962, b), a complete description of the distribution of transit times may require a thorough understanding of the anatomy of the bed under study, including all path lengths, the rheological properties of blood, the driving pressure, the peripheral resistance, the effect of pulsatile flow in an elastic system, the elastic response of the system, and the neural and hormonal factors which affect redistribution of blood along various paths as a function of time (Rossi et al., 1953; Sheppard, 1954; Edwards and Korner, 1958; Thorburn et al., 1959; Korner, 1961). Very little is known about any of these, and the difficulties seem insurmountable, although significant attempts have been made. Stephenson (1948) employed the mathematical approach that a dilution curve is a frequency distribution curve of dye particles, and used Laplace transforms in an attempt to eliminate recirculating indicator. On the grounds that real vascular beds are randomizing nets, too complicated for assessment of the contribution of each of the factors tending to distribute indicator, Sheppard (1954; 1959) has introduced and discussed critically the application of several probability functions, using an indicator-dilution model closely related to that used by Einstein (1905) for the study of Brownian movement. One of the most interesting of these probability functions advanced by Sheppard was the one-dimensional random walk, although it failed to describe indicator-dilution curves in several important details.

Other known probability functions were tested. Sheppard (1952)

originally pointed out that instantaneous injection indicator-dilution curves with a quasi-exponential downslope resemble the normal error-function plotted on a logarithmic horizontal time axis. Stow and Hetzel (1954) pursued this suggestion. They reported reasonably good agreement between curves obtained in the central circulation in man, and those predicted from their equation, although the agreement seems purely fortuitous as far as basic theoretical significance is concerned, as their mathematical model was empirical, being based on a visual resemblance between a log-normal distribution curve and a variety of recorded dye curves. The downlimb of their equation falls more rapidly than Hamilton's simple exponential, so that the area under the curve is smaller, and the flow estimated by the formula consequently greater, although the differences between the two methods, in their hands, averaged only 3.5 per cent.

The work of Stephenson (1948), Sheppard (1954) and Stow and Hetzel (1954) showed therefore that certain well-known distributions such as the Poisson or the log-normal distribution can be fitted to the indicator-dilution curve by appropriate choice of constants. Dow (1956) has pointed out that no definite meaning has been ascribed to these constants in terms of hydraulic factors involved in the dispersion of indicator. In the absence of a rational association, their applicability, in the very situations in which their predictive potential is most needed, is unconvincing.

British workers (Edwards and Korner, 1958; Thorburn et al., 1959; Korner et al., 1960) have specified dispersion in terms of the variance of the indicator-dilution curve by a family of multiple regression equations

of different intercept values. This again was essentially an arithmetical process of improving the fit of the curve without providing much information about underlying hydraulic processes. Korner (1961) has further specified dispersion by integrating the curve to obtain a distribution function from the normal frequency function of the curve. Using the probit transformation, he has demonstrated that the curve is a non-Gaussian distribution function. The introduction of the slopes of the probit regression lines into the multiple regression function specified dispersion for flow and volume with greater accuracy than variance alone, but without more elaborate mathematical analysis the result is still inadequate.

Zierlier (1962, b) is reluctant to accept any of these extrapolations. He feels that, if a method of extrapolation yields a good fit with an observed curve in which recirculation is not prominent, it does not follow that the same distributive law holds in those cases in which recirculation is prominent, in which the accuracy of fit cannot be tested, nor that it holds for other vascular beds. As pointed out by Grodins (1962), every mathematical model is an abstraction, which deliberately neglects certain features of the prototype in order to make possible the rigorous treatment of others. It is not surprising therefore, that "no thoroughly defined system can be expected to mimic completely all the phenomena of physiological importance" (Meier and Zierler, 1954).

CONSTANT INFUSION TECHNIQUES, THEIR PROBLEMS, MODIFICATIONS AND SHORTCOMINGS

The Theory of the Method

Some justification must be offered for the development and validation in this study of an instantaneous injection dye-dilution technique, instead or the original Stewart principle of determining the equilibrium concentration corresponding to a constant rate of indicator infusion. The latter method is based on the principle that when a substance is gradually added to the bloodstream and not removed from the blood within this period, if mixed adequately, the concentration at some point downstream in the vascular system reaches a constant level after a few seconds, from which it is possible to calculate the flow according to the formula $Q = \frac{I}{Cmax.}$, where Q is the rate of blood flow in l./sec., I the rate of dye injection in mg./sec., and Cmax. the concentration of indicator at equilibrium in mg./l. Assuming an adequate plateau level were obtained, this method would be far less subject to errors of calculation, as it entails measurement of only one parameter on the trace, the plateau concentration.

As demonstrated by Hamilton and Remington (1947) and later Lewis (1953, a), the curve from a continuous infusion can be considered as a sequence of instantaneous injections, and is in fact the integral of the curve given by an instantaneous injection. If recirculation appears before the instantaneous injection curve has returned to baseline, then that same recirculation must absolutely prevent attainment of a plateau in a continuous infusion curve; in other words, the curve must begin to rise because of recirculation before it has stopped rising towards the plateau level.

Since recirculation can be detected, and correction made for it in the instantaneous injection curve by semilogarithmic replot, and since no such landmarks are possible on the continuous infusion curve, Hamilton and Remington argue in favour of the instantaneous injection method.

Meier and Zierler (1954) supported and verified the foregoing argument up to the last premise. They pointed out that if the curve from an instantaneous injection is falling along a straight semilogarithmic course, then its integral (the continuous infusion curve), during the same time interval, must be approaching a horizontal asymptote according to the same sort of law. In other wards, the difference between the instantaneous injection concentration and the equilibrium level decreases along a straight semilogarithmic course, exactly parallel to that of the disappearing dye from the instantaneous injection. Theoretically then, constant infusion techniques may yield reliable results if recirculation can be excluded by an analogous, though more complex correction (Meier and Zierler, 1954; Dow, 1956).

Problems in Obtaining a Measurable Plateau Concentration

It is surprising how much controversy has arisen concerning the validity of the method which depends absolutely upon the attainment of a measurable plateau concentration before contamination by recirculating indicator occurs (Wiggers 1944; Holt 1944; Holt et al., 1946; Hamilton and Remington 1947; Rashkind and Morton 1949; Howard et al., 1953; Shepherd et al., 1955). It seems self-evident that the more the injection and sampling sites are approximated, the greater are the chances of

obtaining a longer and uncontaminated plateau section, and it was Holt (1944) who first appreciated this in theory. It is also obvious that in situations with a short plateau section, the use of intermittent sampling techniques would considerably reduce the chances of accurately registering this critical phase of the curve (Howard et al., 1953; Peterson et al., 1954; Shepherd et al., 1955).

Shepherd, Bowers and Wood (1954; 1955) claim to have shown the errors introduced by recirculation by constant infusion into the right ventricle or pulmonary artery, and sampling from the radial artery. Even with catheters thus centrally placed, instantaneous injection dye curves, recorded using the same injection and sampling sites, show that recirculation must be contaminating the constant infusion curve (Howard, Dow and Hamilton, 1951), and that the exact point of this becoming apparent varies with a changing haemodynamic state. Unfortunately, spurious plateaux may be produced by biological instability or phasic flow changes of the respiratory cycle which may give false readings of concentration differences, and so lead to errors in calculation of cardiac output (Howard et al., 1952; 1953; Peterson et al., 1954).

Peterson et al. (1954) overcame the problem of early recirculation obscuring the true plateau by constant infusion into the aortic root, just above the aortic valve, and sampling at a peripheral artery. They offered convincing evidence for adequate mixing of dye with blood within a length of two centimetres in their system, by effective spraying of dye from the orifices of their injection catheter, enhanced by the marked local turbulence and eddying within the aortic root as blood escapes over

the aortic valve. Using this system, they obtained effective plateaux lasting 7 - 10 seconds, compared to plateaux obtained with right ventricular injection of 0 - 2 seconds before recirculation interfered. They showed however, that due to the intermittent nature of flow in the pulmonary artery, no plateau may be apparent, and that with peripheral venous injection, measurable plateaux are unobtainable due to recirculation. Although they claimed that the coronary arteries were perfused with representative dye-blood mixture by their technique, it seems that the coronaries could well receive a non-representative dye-blood mixture with the injection site so near their orifices, which would, of course, invalidate the method. Another objection to the technique, raised by Shepherd et al. (1955), was that arterial catheters in the thoracic aorta adjacent to the semilunar valves sometimes result in emboli formation, a risk which would be quite unjustifiable in view of the proximity of the coronary and cerebral vessels.

Quite obviously the solution to both the foregoing objections is to move the injection site further down into the thoracic aorta, but this brings with it added problems. Firstly, coronary blood flow is not included, as injection is well distal to their orifices, and so total cardiac output is not measured. Secondly, injection velocity must be increased to achieve adequate mixing, once away from the turbulent area above the aortic valve. Grace, Fox, Crowley and Wood (1957) used this method, but found that not only did they have problems obtaining uniform mixing, but that high velocity injection caused a generalised vaso-depressor reaction from the adenosine triphosphate and related substances

released from haemolysed red cells, a problem previously encountered by Andres et al. (1954) in attempting to measure fore-limb flow by a similar technique.

Methods of Measuring Flow Continuously and Indefinitely, and their Problems

Grace's group introduced an ingenious method of "backing off" for recirculating indicator by simultaneous sampling from the abdominal aorta and the left radial artery, the origin of which is proximal to the injection site. This meant that it was possible to measure flow constantly and indefinitely in the presence of recirculating indicator without having to rely on shortlived plateau levels, by subtracting the dye concentration recorded at the left radial artery from that at the abdominal aorta at a corresponding time. This required taking into account the difference in the time needed for the recirculating indicator leaving the left ventricle to reach the two sampling sites. This was obtained by measuring the differences between the peak concentration times of the dilution curves recorded at these sites following instantaneous dye injections into the pulmonary artery, which were carried out just before or just after the constant rate injection into the thoracic aorta. The use of such a constant time correction to correct for recirculating dye introduces a very definite possible source of error, since changes in circulation times to the sampling sites at the radial artery and abdominal aorta might occur during the constant rate injection curve, especially under unsteady state conditions of the circulatory system, for which this method is otherwise ideally suited (Fox, 1962).

Andres et al. (1954) had used a similar principle to plot the linear increase in concentration of recirculating dye, by constructing a line from blood sample dye concentration readings, taken only as frequently as was necessary to establish the slope and intercept of the climbing background dye concentration due to recirculation. Shepherd et al. (1954; 1955) had used a right ventricular catheter during pulmonary artery injection to detect the arrival of recirculating dye, purely as a means of justifying that their constant infusion technique produced adequate plateaux before recirculating dye contaminated them. They, however, were using T-1824, and so encountered problems in pinpointing the appearance time of the recirculating dye exactly, due to fluctuating arterial oxygen saturation interfering with their recordings of absolute concentrations of dye in the pulmonary artery.

Marshall, Fox, Rodich and Wood (1960) introduced a refinement of Grace's technique for continuous thoracic aorta flow recordings. By constant infusion of indocyanine green to determine thoracic aorta flow, as described by Grace et al. (1957), coomassie blue injected instantaneously into the right atrium to measure cardiac output, and with oximeters modified to record the concentrations of the two dyes used in both constant infusion and instantaneous injection simultaneously at their respective spectral absorption peaks in the same oximeter cuvettes, they measured lower body (thoracic aorta) flow continuously, and cardiac output intermittently. Subtraction of the two values yielded a figure for upper body flow (figure 2). Under such circumstances, the simultaneous

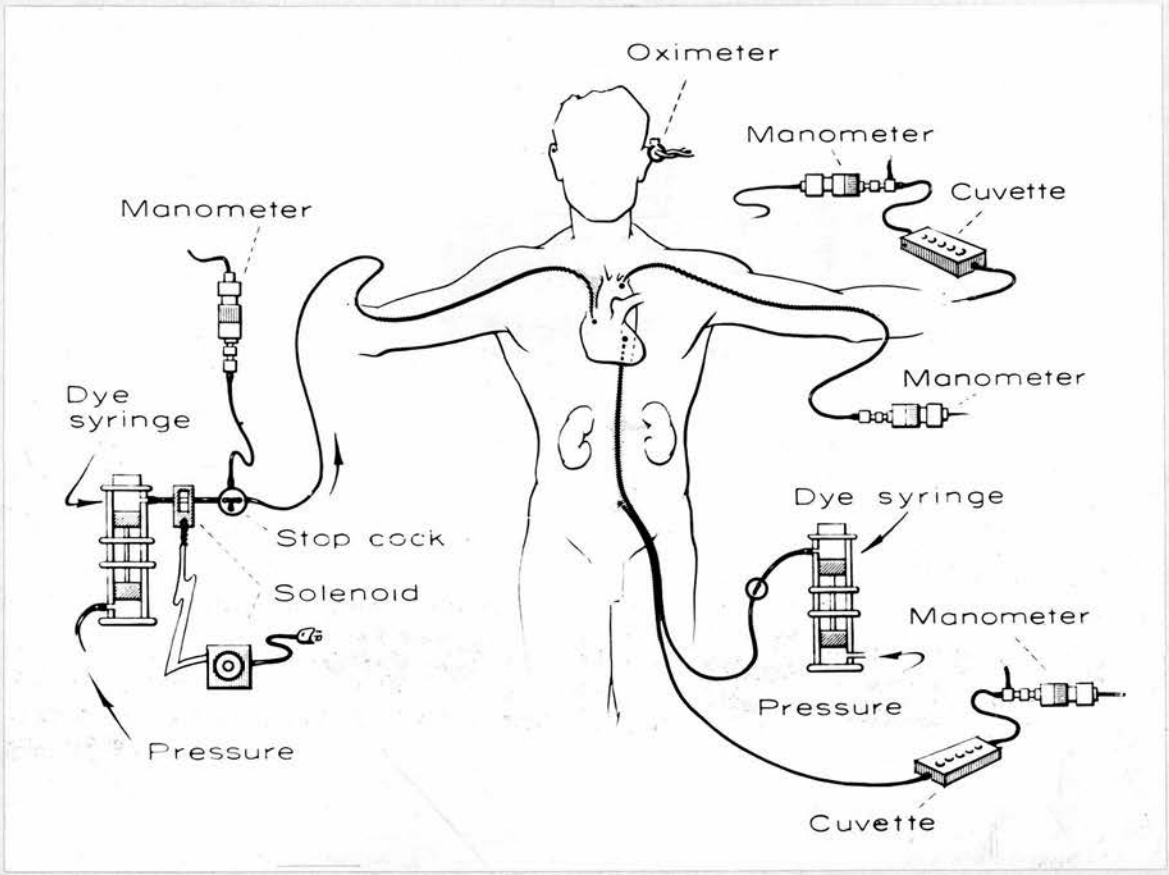

Figure 2: Diagram of assembly for simultaneous recording of total-body blood flow (cardiac output) and lower-body blood flow (thoracic aorta) by instantaneous injection and constant infusion dye-dilution techniques (Fox, 1962)

estimation of cardiac output with coomassie blue, during measurement of thoracic aorta flow with indocyanine green, afforded the appropriate time interval necessary for the correction, referred to earlier, for the amount of recirculating dye being detected at the abdominal aorta at any moment during the constant infusion. In a consideration of the pros and cons of constant infusion techniques however, this is a digression, because it is apparent that the idea of continuous correction for recirculating dye to obtain cardiac output during constant infusion has been explored from several angles, yet in the most refined of all the approaches, that of Marshall et al. (1960) described above, total body flow (cardiac output) is again being measured by instantaneous injection methods. Before dismissing the subject however, what of constant infusion into a mainstem branch of the pulmonary artery, sampling from the aorta or peripheral artery, and sampling from the pulmonary artery root, to back off for the recirculating dye? This was the principle used by Shepherd et al. (1955) to detect the appearance time of recirculating dye. They did not at that time have a dye such as indocyanine green which is not interfered with by oxygen saturation fluctuation. Unfortunately, this seemingly ideal method is open to the same objections as that of Grace et al. (1957) for measuring thoracic aorta flow, namely the inability to apply an appropriate time correction factor during unstable states.

It would be very convenient if an indicator could be found which would not recirculate because of complete removal by the tissues from the

bloodstream. Chidsey, Fritts, Hardewing, Richards and Cournand (1959) and Rochester, Durand, Parker, Fritts and Harvey (quoted by Hamilton, 1962) have infused a solution of radioactive krypton (Kr^{85}) into the right atrium or a peripheral vein and, after it has mixed in the right side of the heart, sampled from the pulmonary artery. It has been shown that this substance appears in low concentrations in arterial blood, and hence returns to the right heart in small and predictable amounts. Right ventricular output can therefore be estimated continuously. Although the concentration of Kr^{85} returning to the right atrium during constant infusion is only about 10 per cent. of the pulmonary artery concentration, this introduces a significant error in the calculation of cardiac output, as no correction is made for recirculating indicator.

The Case against Constant Infusion Techniques

In summary therefore, the points against the use of constant infusion techniques for calculating cardiac output are as follows:

i) The method as originally employed cannot be relied upon to give predictable plateau concentrations.

ii) Use of more recent techniques for correction of recirculating indicator is complicated by the time correction factor for the different catheter sites in changing states. While Marshall et al. (1960) has achieved a satisfactory method of measuring thoracic aorta flow, no satisfactory method for measuring cardiac output by constant infusion techniques has been devised.

iii) Methods for correcting for recirculating indicator require an extra arterial catheter, or right heart catheterization, the latter requiring fluoroscopy, a procedure limiting the versatility of the method.

iv) While the Kr^{85} method represents a conceptual advance, it fails to account for recirculating indicator, as explained, apart from resorting to a radioactive indicator with all its inherent disadvantages.

v) Finally, even in instantaneous injection techniques, the frequency of cardiac output estimates is to some extent limited by the time taken to reinfuse the blood withdrawn during the dye curve inscription. Although a larger withdrawal syringe will allow repeated dye curves, the problem soon arises of clotting in the syringe, with the associated risks of returning such blood to the patient's arterial system. A compromise must therefore be arrived at so that the blood is kept extravascularly for as short a time as possible, while not limiting unduly the duration of withdrawal.

It will be readily seen therefore that the claim for indefinite flow measurement by constant infusion techniques, with twin sampling to correct for recirculating indicator, requires qualification. Duration of flow measurement is limited by the time during which the blood can be

safely kept in the withdrawal syringe before reinfusing it, or by the degree of exsanguination which can be tolerated under the experimental conditions, if the blood is to be discarded. Calculation of the time during which blood is extravascular must be made from the onset of withdrawal, until the last of the syringe's contents returns to the patient. Constant infusion methods of measuring thoracic aorta flow, but not cardiac output, have a definite place in investigative work on condition that their shortcomings are fully appreciated.

THE CALCULATION OF VOLUME FROM INDICATOR-DILUTION CURVES

The Stewart-Hamilton Method

Stewart (1897; 1921, b) suggested that the quantity of blood in the lungs might be estimated on the basis of the indicator-dilution curve, using the formula: $V = \frac{Q}{60} \times T$, where $\frac{Q}{60}$ is the blood flow in ml./sec., T the mean pulmonary circulation time in seconds, and V the volume of blood in the lungs in millilitres. Hamilton's group (Kinsman et al., 1929; Hamilton et al., 1931) further analysed the problem, and agreed with Stewart that the shape of the curve is influenced, not only by flow, but also by the volume of blood in the central pool. Since the pulmonary circulation time could be determined only by animal experiments, they applied the method to humans, using the formula: $V = \frac{Q}{60} \times T_m$, whence V is the volume of the central pool in millilitres, $\frac{Q}{60}$ the flow in ml./sec., and Tm the mean transit time between the site of injection and that of sampling in seconds, as calculated from:

$$Tm = TA + \frac{\int_0^\infty t\, c\,(t)\, dt}{\int_0^\infty c(t)\, dt} = TA + \frac{C_1.T_1 + C_2.T_2 + \ldots . Cn.Tn}{C_1 + C_2 + \ldots \ldots . Cn},$$

where TA is the time of the fastest indicator particle between injection and sampling sites (appearance time), c(t) the concentration of indicator at time t in mg./l., Cn the dye concentration in mg./l., and Tn the number of seconds after the appearance of the fastest moving indicator particle.

Lewis (1953 a) synthesized the continuous infusion curve in an attempt to avoid the use of the mean transit time in the calculation of central blood volume, but his method was in fact simply another way of calculating the mean transit time of Hamilton's group, and merely confirms their method by an alternative derivation.

The accuracy of the estimation of volume by the Stewart-Hamilton equation has been checked in water-filled model systems. The results of the calculated volume agree with direct measurements in models differing widely in their design, and the standard deviation of a single estimate varies from 3 - 5 per cent. (Kinsman et al., 1929; Hoffman and Shillingford, 1957; Thorburn et al., 1959). The only animal experiments providing a direct check on the accuracy of the method were performed by Schlant and colleagues (Schlant, Kraus, Moore, Haynes and Dexter, 1958; Schlant, Novack, Kraus, Moore, Haynes and Dexter, 1959) with Cr^{51}-labelled red cells, which were afterwards measured at autopsy for radioactivity in the homogenized heart and lungs containing the volume in question. Dye curve estimations of volumes performed with Evans Blue were about ten per cent. above the directly measured volumes. This can be explained by the lower haematocrit ratio in the smaller pulmonary blood vessels compared with the large vessel haematocrit, a consideration necessary in their calculation (Dow et al., 1946; Lawson et al., 1952; Rapaport, Kuida, Haynes and Dexter, 1956).

Meier and Zierler (1954) confirmed the validity of the Stewart-Hamilton method of calculating central blood volume, and demonstrated that the vascular system meets the necessary assumptions for the application of the principle. Dow (1956) pointed out that in artificial systems of low randomizing power, which give a small temporal dispersion of the indicator in proportion to its traversal time, only minor errors are caused by faulty choice of a landmark for the mean transit time. But in biological use of

the method, the time scale of the curve itself is of the same order as the appearance time, and proper calculation of the mean transit time is essential. In a symmetrical curve, the mean (average of all transit times), the mode (time co-ordinate of the peak), and the median (time co-ordinate which halves the area) all coincide. But as the curve becomes asymmetrical, and particularly as the terminal portion stretches out much more in time than the initial part, the three measures depart more and more from one another.

Many workers have failed to realise this source of error. Ebert, Borden, Wells and Wilson (1948; 1949) employed the Stewart-Hamilton method of estimating intrathoracic blood volume, but used the median instead of the mean transit time. Nylin and Celander (1950) used the mode in the calculation of volume, as did Beard et al. (1951), although they appreciated that it need not be the same as the true mean transit time. Eliasch (1952) used the median to calculate mean transit time, and Adams (1954) used the mode to calculate central blood volume. More will be said about the shortcomings of the Stewart-Hamilton method for calculating central blood volume, and the limited conclusions which can be drawn from it, as commonly employed, at the end of this section.

The Newman Slope-Volume Method

Kinsman et al. (1929) first observed the exponential decay of a dye curve when recirculation was excluded. They pointed out that this behaviour could be described by the "compound interest law": $C(t) = C_o e^{-\frac{Q}{XV}t}$, mentioned in the section on the theory of the method for calculating flow, where Q represents flowrate, V the volume of the system, and X is a correction

factor to account for incomplete mixing. Because of their uncertainty of the value of X, they concluded that this equation could not yield the value of V. On the contrary, they used it to calculate X, by substituting V obtained from $\frac{Q.Tm}{60}$. Their equation describes a single mixing chamber with instantaneous and homogeneous mixing in a flowing stream, in the total volume XV, but does not describe the first portion of the curve. They realised that their dye was never mixed homogeneously with much of the blood between the sites of injection and sampling, and that the volume corresponding to the known downslope and flowrate was only a small and inconstant fraction of the otherwise indicated total, XV. They could make no headway with the analysis of the earlier parts of the curve and did not pursue the matter.

In 1950, Nylin and Celander derived an identical equation based on theoretical considerations. Although they described a model consisting of three chambers in series (right heart, lungs and left heart), their analysis was limited to the descending portion of the curve, and here they treated the model as though it were a single chamber. Like Hamilton's group, they introduced a factor to account for incomplete mixing, and did not use the slope of the descending limb to calculate V directly, although they did use it to set a lower limit for the range of V. The upper limit for V they derived erroneously by the Stewart-Hamilton method, using the mode instead of the mean transit time. They then took the arithmetic mean of slope-volume and Stewart-Hamilton volume as the best estimate of the heart-lung volume.

The slope-volume method of Newman et al. (1951) for calculating pulmonary blood volume, based on a mixing chamber model, developed out of attempts to find a theoretical model which could account for the form of the indicator-dilution curve. The conditions required for the applicability of their slope principle were emphasised in the initial report on the method. They included instantaneous injection, complete mixing, constant volume, and representative sampling. Unlike Nylin and Celander (1950), they considered the entire curve, and not just the descending limb. They discovered that a three-chambered model plus an arbitrary dead time could generate indicator-dilution curves that resembled those of the prototype fairly well. There was instantaneous homogeneous mixing in each chamber of their model, with chambers whose volume could be varied independently, while no recirculation was allowed. Their equation for a single ideal mixing chamber is identical with that of Hamilton et al. (1932) and Nylin and Celander (1950), except for the omission of the mixing correction, and inclusion of a dead time.

Regarding the first chamber as the analogue of the right side of the heart, a second representingthe lungs and a third representing the left side of the heart may be added. Since later chambers influence neither the flow nor the concentration of the earlier stages, the coupling between them is independent. For the later stages the indicator concentration entering each chamber is not zero, but the output concentration of the previous chamber, and, for the three-chambered model, a set of linear differential equations could be written and solved (Grodins, 1962).

It must therefore be determined how adequately the serial mixing chamber model serves as a theoretical basis for the observed form of the indicator-dilution curve, using the formula $V = \frac{Q}{DS}$ where Q is the flow in volume per unit time, DS is the slope of the downlimb, and V the unknown volume. Newman's group believed that their slope-volume was determined solely by the flow and volume of the lungs alone (Pearce, McKeever, Dow and Newman, 1953), although experimentally it averages 30 - 50 per cent. of the classical Stewart-Hamilton volume in man, and therefore measures only some part thereof, (Nylin and Celander, 1950; Dow, 1955; Shadle, Moore and Billig, 1955; Mills and Kattus, 1956; Wang, Shepherd and Marshall, 1959; 1960). Hetzel, Swan and Wood (1954) calculated central blood volume according to both methods after dye injection peripherally into a vein and centrally into the pulmonary artery. In both instances the estimated central blood volume was smaller after central injection than after peripheral injection. This was to be expected with the Stewart-Hamilton method, but the progressive attenuation of the slopes of the curves according to the injection site contradicts the assertions of Newman's group, that the slope of the dilution curve is governed by the flow and pulmonary blood volume alone.

McGuire, Dock, Hyland, Harrison, Haynes and Dexter (1962) studied the central blood volume by both methods in man with peripheral arterial sampling and injection into the pulmonary artery or left atrium. There was poor agreement between the figures obtained by the two methods. Occlusion of one of the two main branches of the pulmonary artery was reflected in the

values for central blood volume as calculated by the Stewart-Hamilton method, while the slope-volume method showed only minor and less consistent changes in values. They were unable to offer any conclusions about the site of the mixing pool which determined the slope of the curves, which were very similar with both pulmonary artery and left atrial injection. They suggested that their similarity was caused by peripheral sampling, and that a sampling site nearer the lung effluent would reflect the pulmonary blood volume more accurately. Wang et al. (1959; 1960), using similar methods, also found that changes in pulmonary blood volume were not reflected adequately by Newman's method.

Newman's group supported their method by animal experiments (Pearce et al., 1953), although some of their results were disastrous (Grodins, 1962). Their slope-volumes, calculated for pulmonary artery and right atrial injection, were essentially identical, whereas that calculated for pulmonary vein injection was considerably less; this supporting their thesis that the calculated slope-volume lies between that for pulmonary artery and vein injection. Closer examination of the figures reveals that the slope-volume from pulmonary vein to carotid artery averaged approximately 60 per cent. of that from right atrium to carotid artery, contradicting the theory. Marshall, Wang and Shepherd (1960) have produced results pointing to the same discrepancy. Although injections were made into the pulmonary artery and concentrations measured in the left atrium to determine the form of the lung dilution curve, no lung volumes were calculated from these curves for comparison with slope-volumes calculated from curves of right atrial injection and carotid artery sampling.

The most disturbing result of all is the behaviour of the dead time. In the three-chambered model of Newman, all the dead time resides in a delay line located proximal to the entrance of the right side of the heart. Therefore, if indicator is injected anywhere distal to this point, there should be no dead time at all. This does not tally with the actual observations. Sampling from carotid artery there was a dead time of about 1.0 second for aortic root injection, 1.2 seconds for pulmonary vein injection, 3.3 seconds for pulmonary artery injection, and 3.5 seconds for right atrial injection. Obviously the flow net from right atrium to aortic root does not behave as theory demands. This presumably is because the flow net between them (the pulmonary vasculature) does not behave as a perfectly stirred mixing chamber (Dow 1956; Zierler 1962 b).

Lung perfusion experiments have not given exponential washout curves, unlike experiments which include the heart in the circuit (Hamilton, 1953). Lung perfusion curves simulate those obtained when laminar flow was studied in artificial circulatory systems (Schambye 1953; Rossi et al., 1953).

Parrish et al. (1959), applying dye curves obtained from dogs in the construction analysis of an electronic analogue simulator, also suggested that blood flow in the lungs is of a laminar type rather than a single mixing pool type with perfect and instantaneous mixing.

The slope-volume then measures some fraction of the classical volume, but its anatomical boundaries are indefinite. Sheppard (1954) concludes that the general resemblance between theoretical&experimental curves is the result of a statistical accident.

Discussion of the Methods

Both methods for calculating central volume are based on the same fundamental assumptions discussed under the mathematics of calculating flow. If flow and volume are not constant during the determination, the exponential downslope may be distorted, so that the semilogarithmic extrapolation is not possible, and the Stewart-Hamilton formula cannot be used for calculating cardiac output, and so central blood volume. Generally however, curves do have exponential downslopes, as discussed in the section under the theory of the indicator-dilution method, although this can hardly be taken to show that these assumptions hold. It may be the result of laminar streaming in the vessels leading the dye-blood mixture to the sampling site, so that possible deviations from the exponential washout are smoothed out.

Instantaneous injection is impossible and must take a finite time. If the mean transit time through the system is long compared to the mean time of injection, the fact that the injection is not instantaneous can be ignored (Hoffman and Shillingford, 1957). As has already been pointed out however, unduly prolonged injection can cause "contamination" of the downslope by recirculating indicator before the curve has attained an exponential decay. This will invalidate the measurement of flow, necessary in the calculation of volume. More important however, is the fact that, if the mean time of the distribution of the injectate is significant compared to the mean transit time through the system, the equation for the calculation of mean transit time, and therefore volume, will be erroneous (Zieler, 1958; 1962 b).

Obviously, an effect on the curve produced by the sampling-detecting recording system will distort the dye curve, but should not alter its area. The distortion will however affect estimations of appearance and mean transit times, and corrections for these must be made in their calculation according to the system used. The correction in the present system, as calculated from the regression equations of Milner and Jose (1960) are 0.83 seconds for appearance time and 0.82 seconds for mean circulation time.

The Bradley Method: Because of the extreme variability in the lengths of the vascular segments traversed by indicator in transit through the lungs, it seems possible that, if sequestration should occur in certain of the longer vascular pathways, so that their dye-blood content reaches the sampling site too late to be measured during the first circulation, the calculated curve area will be too small, and volume therefore over-estimated. To account for this possibility, Rabinowitz and Rapaport (1954) suggested Bradley's method of measuring organ blood volume (Bradley, Marks, Reynell and Meltzer, 1953). In this method the total venous drainage of an indicator from an organ is subtracted from the total arterial input of the indicator to give the net amount left in the circulatory bed in question, up until the time the arterial and venous concentrations in the blood in the organ equilibrate.

$$V = \frac{Q \times T_{eq.} \times (\bar{c}_{in} - \bar{c}_{out})}{C_{eq.}}$$

In the above formula V is the blood volume of the organ in millilitres, Q the flow through the organ in ml./min., Teq. the time required for equilibrium

of indicator concentration in minutes, $\bar{c}_{in}$ and $\bar{c}_{out}$ the mean concentrations of indicator in mg./ml. entering and leaving the organ respectively before equilibrium, and Ceq. the equilibrium concentration in mg./ml. Differences between $\bar{c}_{in}$ and $\bar{c}_{out}$ vary directly as the volume of the organ, and inversely as the rate of volume flow by this method. Hence the difference will be wide when the volume is large and the flowrate small. When, on the other hand, volume is small and flowrate large, the difference is reduced, and small errors in their determination will have a marked effect on calculated volume. The method is suitable for volume measurements in vascular beds only where the ratio of flow to volume has a value greater than four, and is therefore unsuitable for pulmonary blood volume estimation (Braunwald, Fishman and Cournand, 1958), apart from the practical consideration that sampling is necessary at both entrance and exit sites. Braunwald et al. (1958) showed this quite conclusively in model experiments using the Stewart-Hamilton and Bradley methods simultaneously. They also showed that the Stewart-Hamilton method gave an accurate measure of volume over a wide range of volume-flow ratios.

Attempts to Partition the Central Blood Volume: The volume calculated by the Stewart-Hamilton method is difficult to visualise anatomically. It includes the vessels and heart chambers actually traversed by the indicator, plus other arterial branches temporally equivalent to the sampled one, plus, in the case of peripheral venous injection, temporally equivalent venous tributaries. Since it is pulmonary blood volume which is of greatest interest, attempts have been made to partition this rather vague volume.

Lagerlöf, Werkö, Bucht and Holmgren (1949) injected indicator into the pulmonary artery and sampled from the brachial or femoral artery. From the calculated central blood volume they subtracted half the heart volume, determined by the method of Liljestrand, Lysholm, Nylin and Zachrisson (1939), and the aortic blood volume, calculated according to the method of Gidlund and Porjé (1948), thus obtaining the approximate pulmonary blood volume. Nylin and Celander (1950) similarly subtracted the roentgenologic approximations of heart volume according to the same method to partition off this fraction of calculated central blood volume into the right ventricular, pulmonary vascular, and left ventricular components with an indirect mathematical model based on the mixing chamber model of Newman et al. (1951), which, as has already been discussed, is theoretically unsound. Milnor and Bertrand (1954) showed a more imaginative approach with multiple sites of injection and sampling in studies on dogs, though their work is open to certain theoretical objections implicit in the assumptions necessary for the validity of the Stewart-Hamilton method of measuring volume. Similar principles were used by Bing, Heimbecker and Falholt (1951), Holt (1956), and Holt and Allensworth (1957), although their primary objectives were to measure the residual volume of the ventricle. These approaches however may be assumed to have paved the way to the best indicator-dilution method of measuring actual pulmonary blood volume at present available which will be discussed at the end of this section.

The Interpretation of the Stewart-Hamilton Value for Central Blood Volume: What interpretation can therefore be placed on central blood volume as measured in the present study, with injection into the right heart or pulmonary artery and sampling from the aortic root? Numerous workers have drawn conclusions about the changes in intrathoracic blood volume during various states of rest, posture, exercise, etc., and in varied pathological states, on the basis of this rather ill-defined volume. Accepting its lack of absolute anatomical definition however, limited conclusions can be drawn, although their interpretation is difficult. Any increase in intrathoracic blood volume may be interpreted as being due to an increase in the blood in the pulmonary vascular bed, with an unchanged volume in the heart chambers, or a relatively smaller increase in both, or an increase in the heart volume alone, or an increase in one of the above, sufficiently large to mask a concomitant decrease in the other. With the above reservations, the values for central blood volume have been calculated in the present study, corrected for distortion of mean transit time by the sampling-detecting-recording system.

The validity of central blood volume is open to even more serious objections during a change of physiological state. As has been explained, central blood volume has been measured according to the Stewart-Hamilton formula, $V = \frac{Q}{60} \times Tm.$ and the value for Q by indicator-dilution methods will not differ according to the sampling site. The other variable, mean transit time (Tm), may however change by differing amounts if measured in different vessels, if the change in flow (Q) is not equally distributed to the various parts of the body. This point has been most elegantly

demonstrated by Gleason, Bacos, Miller and McIntosh (1959), Marshall (1960), and Marshall and Shepherd (1961). If a subject performs heavy leg exercise, the cardiac output rises, but the increased flow is distributed mainly to the exercising legs, while flow to the non-exercising arms is not similarly increased. If then the dye curve is inscribed from a catheter sampling from the radial artery, the flow increase will be faithfully represented, but the mean transit time, consisting largely of appearance time, may hardly alter. Another catheter sampling from the femoral artery however, would reveal a considerably shortened mean transit time compared to the pre-exercise value. The comparison of resting and exercising central blood volumes (Q/60 x Tm), as derived from the arm, will therefore be considerably different from those derived from the leg. Marshall and Shepherd (1961) were able to achieve a figure for central blood volume which was so erroneous that it actually exceeded the predicted total blood volume, by peripheral venous injection and radial artery sampling during heavy leg exercise, simply because the mean transit time change in the curve from the radial artery was not representative of the entire arterial system. They went on to explain that false increases in central blood volume, found by the above method during exercise, are partly or wholly caused by an increase in the systemic "arterial" component of the central blood volume, in consequence of relative changes in the rates of blood flow to different regions of the body. Much of the confusion has arisen from attempts to think of the central blood volume as a conventional three-dimensional volume. Its limits, however, do not conform to any fixed anatomical boundaries, but are determined by equivalence in the fourth

dimension, namely time. The elusive nature of such a volume is attested to by the number of synonyms which have been used to describe it, including the "intrathoracic", "cardiopulmonary", "central", and "needle-to-needle" volume, none of which is satisfactory (Dow 1956). Failure to appreciate the significance of such haemodynamic changes during exercise (Doyle et al., 1953; Kaufman, 1957; Mitchell, Sproule and Chapman, 1958; Thompson, Berry and McIntosh, 1959; Roncoroni, Aramendia, Gonzalez and Taquini, 1959; Braunwald and Kelly, 1960), changes in posture (Marshall, 1959; Weissler, Leonard and Warren, 1959), general anaesthesia (Johnson, 1951; Lee, Churchill-Davidson, Miles and de Wardener, 1953; Etsten and Li, 1954), in heart disease (Kopelman and Lee, 1951; Ball, Kopelman and Witham, 1952; Doyle, Lee and Kelley, 1952; Doyle et al., 1953; Kattus, Rivin, Cohen and Sofio, 1955; Rapaport et al., 1956) and in the interpretation placed upon the reservoir function of the lungs in cardiac output (Johnson, 1951; Sjöstrand, 1953; Warren, Weissler and Leonard, 1959; Weissler et al., 1959) have led in the past to unwarranted conclusions about changes in actual pulmonary blood volume, the reservoir function of the lungs, and the interdependence of pulmonary blood volume and cardiac output. Valid information of changes in the pulmonary blood volume cannot be obtained by measurement of the central blood volume when a peripheral sampling site is used (Marshall et al., 1960). To quote Marshall and Shepherd (1961): "It is difficult to justify any further studies on such a nebulous entity as the central blood volume as it usually has been measured."

With a sampling catheter tip in the aortic root the non-representative behaviour of the mean transit time can be overcome, and in the present study this was the policy adopted. The problem remains, however, of exactly what volume was measured by the method, since it embraces heart chambers and pulmonary vascular bed, and for this reason the results of the measurements of central blood volume have not been interpreted.

Methods for Obtaining Pulmonary Blood Volume

A technique has recently been evolved to measure the volume of the pulmonary vascular bed. The method unfortunately requires left atrial catheterization, a procedure which is difficult to justify at the present time in physiological studies in normal subjects, where its application would be invaluable. Two very similar methods have been employed. Milnor, Jose and McGaff (1960) injected indocyanine green into the pulmonary artery and sampled from a peripheral artery. Shortly after, they repeated the dye curve, injecting this time into the left atrium. Assuming that flow remains constant during the consecutive measurements, the mean transit time from pulmonary artery to left atrium (the pulmonary circulation time) can be derived by subtraction of the mean transit time from left atrium to brachial artery, from the mean transit time from pulmonary artery to brachial artery: $Tm^{PA-LA} = Tm^{PA-BA} - Tm^{LA-BA}$. Dock, Kraus, McGuire, Hyland, Haynes and Dexter (1961) and McGuire et al. (1962) modified this slightly by simultaneous injection of two different indicators (Evans blue and radioactive iodinated serum albumin), one into the pulmonary artery, and the other into the left atrium, with peripheral arterial sampling.

This overcame the problem of flow changes between estimates for investigation in a changing state, but it required the rather retrograde step of reversion to intermittent sampling, as the sampled blood needs separate techniques for estimation of each indicator concentration. The use of indocyanine green and coomassie blue instead of Evans blue and radioactive iodinated serum albumin with a modified oximeter circuit, as employed by Marshall et al. (1960) in their simultaneous measurements of cardiac output and thoracic aorta flow, would overcome this drawback, allowing continuous recording of both indicator concentrations simultaneously. Quite obviously, sampling from the left atrium would require only one indicator, which would simplify the method considerably, but, apart from being cumbersome and requiring a long catheter with a limited bore, thus giving very poor volume-flow ratio characteristics, there is considerable doubt whether indicator, injected into pulmonary artery and sampled from the left atrium, would undergo complete mixing, since it does not traverse a ventricular chamber (v.d. Feer 1958; Wang et al., 1959). The two-indicator technique, which arrives at pulmonary circulation time by subtracting the mean transit times of the two indicator-dilution curves, probably also suffers from similar shortcomings. The calculation of mean transit time requires a reliable indicator dilution curve, for which one of the requirements is instantaneous mixing of injected indicator. As McGaff, Jose and Milnor (1959) pointed out, there is evidence that complete mixing does not occur in the left atrium (Silver, Kirklin and Wood, 1956; Swan, Burchell and Wood, 1954), in addition to which control of the catheter tip in the left atrium is difficult, and indicator may be injected through the mitral valve, regurgitate into the pulmonary veins, or become sequestered in the atrial appendix.

A SURVEY OF PREVIOUS COMPARISONS OF THE FICK AND INSTANTANEOUS INJECTION DYE-DILUTION METHODS IN MAN

At the present time, the most reliable and precise methods of measuring the cardiac output in man are based upon either the Fick principle or the indicator-dilution technique. Other less valuable methods require the use of empirical constants which must be established by calibrating against one or other of the more reliable methods. The direct Fick method is more firmly established than the indicator-dilution method, and it would seem only natural therefore that the most valid method of assessing the accuracy of the indicator-dilution technique would be by comparing it with the direct Fick, rather than with other available methods.

Since the pioneer comparison of the two methods in six dogs by Moore et al. (1929), numerous attempts have been made to demonstrate the accuracy of the indicator-dilution method in man and in animals by comparison with the direct Fick method. As mentioned earlier, the joint paper published by Hamilton's group in Georgia and Cournand's team in New York (Hamilton et al., 1948), and the similar study by Werkö et al. (1949) the following year, comparing the Fick and dye-dilution methods in man, were landmarks in the acceptance of the indicator-dilution technique. The great number of similar studies which have been reported since, however, testify to the fact that in many of these there has been substantial disagreement between the two methods. In the course of this study, an attempt will be made to show the reasons for these failures, and to suggest how such errors may be reduced, although, for reasons which will emerge, it is quite obvious that absolute agreement is impossible in view of the

inherent differences in the two methods of the time period over which flow is measured. In a later section, a comparison of the two methods will be described, in which experimental conditions will be rigorously controlled to avoid, as far as is practicable, the sources of error which have dogged previous investigators.

Previous comparisons of the two methods have been performed in both animals and in man, using constant infusion and instantaneous injection techniques, and by dye-dilution and other indicator-dilution methods. To reduce a vast array of literature to manageable proportions, and because it is the accuracy of a dye-dilution method in man, and not any indicator-dilution technique which is to be demonstrated in this study, only the comparisons in man using dye as an indicator with instantaneous injection will be considered. The results of these studies will be analysed by the same statistical method as those of the present study in a later section, wherever the data presented make such a procedure possible.

The sources of error in previous studies are of two types:

i) Those due to the inferior techniques available at the time of the study.

ii) Those due to poor planning of the investigation.

Both these groups account for a considerable proportion of the errors; the former group is certainly not a fault of the workers themselves, and the present study has all the advantages which modern instrumentation brings. This will be described under the discussion of the components of the system used in the present study, and comprehensive reasons for the undesirability

of certain technical aspects will not therefore be given at this stage. Notable examples however, are those of the method of sampling and the dye used. Intermittent sampling inevitably reduced the accuracy with which dye curves could be inscribed, whereas present continuous recording instruments have largely overcome these errors. The use of intermittent sampling severely limited the number of observations which could be made per subject because of the associated blood loss. This limitation reflected itself in the plan of investigation where only one dye curve per Fick was practicable for comparison. Evans blue, by far the most widely used dye in such comparisons, had many disadvantages which will be discussed elsewhere. The major ones in this context were the limitation it imposed on the number of observations cosmetically possible in any one subject, and the frequency with which dye curves could be repeated due to the method of calibration once continuous recording techniques became available. It, therefore, also indirectly affected the plan of the investigation, as did intermittent sampling.

The severest criticism which can be levelled at previous studies, however, is the asynchrony of the two methods under comparison. Expired air collections for the determination of oxygen uptake were frequently made some time after the dye-dilution curves and blood sampling. This could have been improved upon and was not a reflection of the techniques available. The stability of the subject was not always ensured, which must have caused errors in the Fick results. Errors in the Fick method due to the variability of mixed venous blood oxygen saturation were difficult to overcome by more frequent sampling because of the tedious methods then available

for the determination of oxygen saturation. They could not, however, have rivalled the time-consuming task of analysis of individual blood samples obtained from a single dye curve using intermittent sampling.

Perhaps the greatest source of error, especially in an unstable patient, was the inevitable inaccuracy resulting from comparison of a single dye-curve cardiac output, measured over a period of approximately a quarter minute, with a Fick cardiac output which gave a mean value over several minutes. Until the advent of continuous recording, and latterly indocyanine green, this was unavoidable for reasons mentioned above. It would, however, be expected that the average of more frequent dye curves during a Fick period would more closely agree with the Fick value, and this has been practised in the present study.

To avoid tedious repetition, the relevant 24 dye Fick comparisons to date have been assembled with available details of the subjects studied and the methods used, with appropriate comments on each. Where adequate data are given, the results have been analysed and presented in a later section, together with those of the present study.

Hamilton, Riley, Attyah, Cournand, Fowell, Himmelstein, Noble, Remington, Richards, Wheeler and Witham (1948).

Number of observations:	46
Number of subjects studied:	31
Types of subjects studied:	5 normal subjects 24 patients with heart or lung disease, ten of whom suffered from compensated or decompensated cardiac failure 1 syphilitic without cardiovascular involvement 1 chronic alcoholic
State of subjects studied:	39 resting 7 exercising
Dye used:	Evans blue
Injection site:	R.A., R.V. or antecubital vein
Sampling site:	Brachial artery
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	In some studies, 1½ minute expired air collections; in others, oxygen consumption was measured over 10 - 15 minutes using oxygen-filled B.M.R. spirometer
Mixed venous blood samples:	Taken over period of one minute from R.A., R.V. outflow tract or P.A.

Comments: Dye and Fick estimations were asynchronous, but were "within a minute or two" or each other.
Comparisons during exercise were not analysed separately.
In view of the site of mixed venous sampling, not all samples can be taken as truly representing mixed venous blood.

Werkö, Lagerlöf, Bucht, Wehle and Holmgren (1949).

Number of observations:	66
Number of subjects studied:	50
Types of subjects studied:	6 normal subjects
	18 hypertensives
	12 patients with mitral valve disease
	6 pregnant women
	2 patients with pulmonary diseases
	6 patients with either arteriosclerotic heart disease or congenital heart disease (unspecified)
State of subjects studied:	All studied at rest; 16 studied after administration of either cedilanid, theophylline or dehydroergotamine
Dye used:	Evans blue
Injection site:	P.A.
Sampling site:	Brachial artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	2 minutes
Mixed venous blood samples:	Single samples from P.A.

Comments: Dye curve, and arterial and mixed venous samples taken during the expired air collection.
Comparisons after drug administration not analysed separately.
An erroneous correction for the measurement of the injectate delivered was made in all studies, leading to falsely high dye cardiac output values (Eliasch, Lagerlöf, Bucht, Ek, Eriksson, Bergstrom and Werkö, 1954).

Friedlich, Heimbecker and Bing (1950).

Number of observations:	9
Number of subjects studied:	9
Types of subjects studied:	No information given
State of subjects studied:	No information given
Dye used:	Evans blue
Injection site:	Not specified
Sampling site:	Not specified
Sampling method:	Continuous recording. Blood withdrawn by vacuum suction via mercury receptacle
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	No information given

Comments: No information given on time relationships of Fick and dye determinations of cardiac output.

Obtained a fair correlation only when a six per cent. correction was added to cardiac output values obtained by dye-dilution to correct for coronary blood flow; this is theoretically unsound, and without it the dye values were systematically lower than the Fick.

Beard and Wood (1951).

Number of observations:	**12**
Number of subjects studied:	**12**
Types of subjects studied:	**Patients with cardiac or respiratory disease without a cardiac shunt**
State of subjects studied:	**Not specified**
Dye used:	**Evans blue**
Injection site:	**Antecubital vein**
Sampling site:	**Radial artery**
Sampling method:	**Continuous recording. Vacuum withdrawal of blood**
Number of dye curves per Fick:	**1**
Duration of oxygen uptake determination:	**Not specified**
Mixed venous blood samples:	**No information given**

Comments: "Almost simultaneous" comparisons of dye and Fick values for cardiac output.

Johnson (1951).

Number of observations:	130
Number of subjects studied:	49
Types of subjects studied:	"Surgical" patients
State of subjects studied:	At rest, after premedication for an anaesthetic, and during anaesthesia
Dye used:	Evans blue
Injection site:	P.A.
Sampling site:	Brachial artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	2 - 3 minutes
Mixed venous blood samples:	Taken from P.A. during oxygen uptake

Comments: In 103 comparisons the Fick and dye estimations were performed within three minutes. In 27 cases the oxygen uptake was estimated asynchronously with the withdrawal of blood samples

It is doubtful whether the subjects were in a steady state when comparisons were made under anaesthesia with a nitrous oxide and oxygen gas mixture.

Kopelman and Lee (1951).

Number of observations:	28
Number of subjects studied:	28
Types of subjects studied:	7 normal subjects 1 nephritic 1 bronchitic Remaining patients suffered from varying combinations of the following disorders: Hypertension (6) Cardiac failure (4) Aortic stenosis (4) Mitral stenosis (12) Atrial fibrillation (12) Bronchitis (1)
State of subjects studied:	At rest
Dye used:	Evans blue
Injection site:	P.A.
Sampling site:	Brachial artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	Taken before and after dye curve from P.A.

Comments: Time relationship of oxygen uptake and dye curve not stated.

Nicholson and Wood (1951).

Number of observations:	8
Number of subjects studied:	8
Types of subjects studied:	Patients with unspecified cardiovascular abnormalities
State of subjects studied:	No information given
Dye used:	Evans blue
Injection site:	Antecubital vein
Sampling site:	Radial artery
Sampling method:	Continuous recording. Vacuum withdrawal of blood
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	No information given

Comments: Four of the comparisons were not simultaneous, and four were almost simultaneous.

The dye curves were abnormal because of the nature of the cardiovascular disorders. The extrapolation of the downslopes was made empirically, regardless of the time relationships or the lack of evidence for systemic recirculation of the dye. Such a procedure is not mathematically justifiable on "abnormal" curves.

Eliasch (1952).

Number of observations:	66
Number of subjects studied:	48
Types of subjects studied:	All patients with mitral stenosis (Grade I - IV) in sinus rhythm or with atrial fibrillation
State of subjects studied:	47 resting
	19 exercising
Dye used:	Evans blue
Injection site:	P.A.
Sampling site:	Brachial artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	P.A.

Comments: Resting, Fick and dye determinations were simultaneous, but the time relationships of the dye curve, expired air collection and blood sampling are not stated. The timing of exercising values for Fick and dye determinations are not stated.

Author subdivided his results before analysis, according to the severity of the mitral lesion, and according to the cardiac rhythm, but not according to whether the patients were at rest or exercising.

Author neglected "trapped" plasma in his haematocrit figures, causing dye values for cardiac output to be slightly too high (Dow, 1956).

He employed an erroneous correction for the measurement of the injected dye in all studies, thus giving unduly high dye cardiac output values (Eliasch et al., 1954).

Omhori, Sasamoto and Hosono (1952).

Number of observations:	13
Number of subjects studied:	13
Types of subjects studied:	Patients with pulmonary tuberculosis
State of subjects studied:	Not specified
Dye used:	Evans blue
Injection site:	P.A.
Sampling site:	Femoral artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	No information given

Comments: No details given of time relationships of Fick and dye studies. No details of Fick method given.

Doyle, Wilson, Lepine and Warren (1953).

Number of observations:	152
Number of subjects studied:	78
Types of subjects studied:	53 normal subjects
	14 patients with congestive cardiac failure
	6 patients with mitral stenosis
	5 patients with tricuspid stenosis
State of subjects studied:	At rest
Dye used:	Evans blue
Injection site:	P.A.
Sampling site:	Brachial artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	3 minutes
Mixed venous blood samples:	No information given

Comments: The Fick estimation was made after the dye curve. "In most cases" the oxygen uptake was measured at the same time as the blood samples for the Fick were taken.

Dow (1956) claims that they had recognised, but unidentified, technical troubles.

Warner and Wood (1953).

Number of observations:	25
Number of subjects studied:	15
Types of subjects studied:	Patient with clinically severe mitral stenosis
State of subjects studied:	Not specified
Dye used:	Evans blue
Injection site:	Not specified
Sampling site:	Radial artery (cuvette oximeter)
Sampling method:	Continuous recording by ear oximeter and cuvette oximeter. Vacuum withdrawal of blood through cuvette oximeter
Number of dye curves per Fick:	One ear oximeter curve per Fick, and one cuvette oximeter curve per Fick in twelve instances
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	No information given

Comments: "Almost simultaneous" dye and Fick estimations of cardiac output. Dye cardiac outputs obtained by ear oximeter were measured by "triangle" method, which is known to increase the inherent variability of the dye method from approximately 11 per cent. to 14 per cent. in the hands of the authors (Warner and Wood, 1953).

Eliasch, Lagerlöf, Bucht, Ek, Eriksson, Bergstrom and Werkö (1954).

Number of observations:	352
Number of subjects studied:	209
Types of subjects studied:	109 patients with mitral valve disease in whom 181 studies were performed
	50 hypertensive patients in whom 90 studies were performed
	14 patients with aortic valve disease in whom 25 studies were performed
	36 subjects without cardiopulmonary disease in whom 56 studies were performed
State of subjects studied:	At rest
	Exercising
	After administration of a drug
	After a change of posture
Dye used:	Evans blue
Injection site:	P.A.
Sampling site:	Brachial artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determinations:	2 minutes
Mixed venous blood samples:	Single samples from P.A.

Comments: Synchronous dye and Fick determinations of cardiac output, and blood samples for Fick estimation taken during period of expired air collection.

Results were subdivided by authors according to the groups of subjects described above.

Gilmore, Hamilton, Kopelman and Sommer (1954).

Number of observations:	25
Number of subjects studied:	25
Types of subjects studied:	Patients with a variety of cardiac disorders other than congenital heart disease
State of subjects studied:	Not specified
Dye used:	Evans blue
Injection site:	R.A. or P.A.
Sampling method:	Ear oximeter
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	No information given

Comments: Dye and Fick cardiac outputs said to be simultaneous.

Neely, Wilson, Milnor, Hardy and Wilson (1954).

Number of observations:	31
Number of subjects studied:	22
Types of subjects studied:	Surgical patients without cardiopulmonary disorders
State of subjects studied:	At rest
Dye used:	Evans blue
Injection site:	In about two-thirds of the patients dye was injected into a large arm vein, and in about one-third into P.A.
Sampling site:	Femoral artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	Two samples taken from P.A. per oxygen uptake

Comments: Dye and Fick cardiac outputs not performed simultaneously, but immediately consecutively. Fick blood samples taken within period of measurement of oxygen uptake while patient breathed pure oxygen from B.M.R. machine.

Smith, Wikler and Fox (1954).

Number of observations:	19
Number of subjects studied:	18
Types of subjects studied:	Patients had variety of unspecified cardiovascular disorders
State of subjects studied:	At rest
Dye used:	Evans blue (or I^{131} -labelled human serum albumin)
Injection site:	Antecubital vein or femoral vein
Sampling site:	Femoral artery
Sampling method:	Intermittent sampling
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	No information given

Comments: Fick and dye determinations said to be simultaneous.

Korner and Shillingford (1955).

Number of observations:	18
Number of subjects studied:	Not specified
Types of subjects studied:	Not specified, but some had rheumatic valvular disease of various types
State of subjects studied:	Not specified
Dye used:	Evans blue
Injection site:	Not specified
Sampling method:	Ear oximeter
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	No information given

Comments: No information on whether dye and Fick estimations were simultaneous.

Shepherd, Bowers and Wood (1955).

Number of observations:	21
Number of subjects studied:	21
Types of subjects studied:	10 normal subjects
	4 patients with mitral stenosis
	3 patients with coarctation of the aorta
	1 patient with aortic incompetence
	1 patient with essential hypertension
	1 patient with pulmonary fibrosis
	1 patient who had had an aorto-pulmonary window closed surgically
State of subjects studied:	17 at rest
	4 exercising
	1 during anaesthesia
Dye used:	Evans blue
Injection site:	R.V. or P.A.
Sampling site:	Radial artery
Sampling method:	Continuous recording. Vacuum withdrawal of blood
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	R.V. or P.A. samples

Comments: Dye and Fick cardiac output determinations not synchronous, but average of six minutes between them.

Falholt and Fabricius (1956).

Number of observations:	18
Number of subjects studied:	18
Types of subjects studied:	Normal subjects, patients with mitral valvular disease, coronary artery disease and "others"
State of subjects studied:	Not specified
Dye used:	Evans blue
Injection and sampling sites:	P.A. injection with femoral artery sampling in 15 subjects R.V. injection with P.A. sampling in 3 subjects
Sampling method:	Continuous recording. Arterial pressure head used to drive blood through cuvette when sampling from femoral artery; vacuum withdrawal of blood when sampling from P.A.
Number of dye curves per Fick:	One dye per Fick in 6 subjects Two dyes per Fick in 12 subjects
Duration of oxygen uptake determination:	3 minutes
Mixed venous blood samples:	Taken from P.A. during oxygen uptake measurements

Comments: Where two dye curves per Fick were performed, the second dye curve was started at the end of the Fick estimation.

Right ventricular dye cardiac outputs cannot theoretically be compared with the Fick in the same study as left ventricular dye cardiac outputs.

Complete mixing of dye before sampling is questionable when injected into R.V. and sampled from P.A. Moreover, the validity of dye curves recorded from P.A. is doubtful when Evans blue is used, because of the interference of fluctuating concentrations of reduced haemoglobin with continuous recording.

Fritts, Harris, Chidsey, Claus and Cournand (1957).

Number of observations:	**13**
Number of subjects studied:	**9**
Types of subjects studied:	**Normal subjects**
State of subjects studied:	**At rest**
Dye used:	**Evans blue**
Injection site:	**R.A.**
Sampling site:	**Brachial artery**
Sampling method:	**Continuous recording**
Number of dye curves per Fick:	**1**
Duration of oxygen uptake determination:	**Not specified**
Mixed venous blood samples:	**P.A.**

Comments: **Dye and Fick estimations not simultaneous.**

Hetzel, Swan, Ramirez de Arellano and Wood (1958).

Number of observations:	36
Number of subjects studied:	31
Types of subjects studied:	15 normal subjects 16 patients without cardiovascular abnormalities on cardiac catheterisation
State of subjects studied:	At rest
Dye used:	Evans blue
Injection site:	14 estimations - main pulmonary artery 8 estimations - left pulmonary artery 8 estimations - superior vena cava 6 estimations - right pulmonary artery
Sampling site:	Radial artery
Sampling method:	Continuous recording. Vacuum withdrawal of blood
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	No information given

Comments: Fick and dye estimations were within 1 - 20 minutes of each other.

Sekelj, Bates, Johnson and Jegier (1958).

Number of observations:	28
Number of subjects studied:	14
Types of subjects studied:	9 patientswith pulmonary stenosis 1 patient with mitral stenosis 1 patient with aortic stenosis 1 patient with mitral incompetence 1 patient with pulmonary hypertension 1 patient with cor pulmonale 9 of these subjects were children aged three to nine years of age
State of subjects studied:	At rest
Dye used:	Evans blue
Injection site:	R.V. or P.A.
Sampling method:	Ear oximeter
Number of dye curves per Fick:	1
Duration of oxygen output determination:	Not specified
Mixed venous blood samples:	P.A.

Comments: Dye and Fick estimations were nearly simultaneous.

Authors admit to technical difficulties of measuring the oxygen uptake in heavily sedated child subjects. Two comparisons from the same subject were excluded from their analysis of results because of suspect Fick values, due to an unstable oxygen uptake in successive determinations.

Authors used an empirically derived constant for calibrating all dye curves.

Richardson, Wyso, Hecht and Fitzpatrick (1959).

Number of observations:	74
Number of subjects studied:	48
Types of subjects studied:	Patients with cardiovascular disease of functional classification I - III
State of subjects studied:	At rest
Dye used:	Evans blue or indocyanine green
Injection site:	P.A. (54 studies)
	Antecubital vein (20 studies)
Sampling site:	Brachial artery
Sampling method:	Continuous recording. Falling-mercury sampling system
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	5 - 6 minutes
Mixed venous blood samples:	One minute P.A. samples in middle of measurement of oxygen uptake

Comments: Dye and Fick estimations were nearly simultaneous. The comparisons with P.A. injection were analysed separately from those with peripheral venous injection of dye.

Taylor and Shillingford (1959).

Number of observations:	53
Number of subjects studied:	26
Types of subjects studied:	Subjects studied suffered from unspecified rheumatic heart disease, thyrotoxicosis, or essential hypertension
State of subjects studied:	At rest
Dye used:	Coomassie blue
Injection site:	P.A.
Sampling method:	Ear oximeter
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	Not specified
Mixed venous blood samples:	P.A. samples taken during oxygen uptake determination

Comments: Simultaneous dye and Fick determinations of cardiac output.

Miller, Gleason and McIntosh (1962).

Number of observations:	34
Number of subjects studied:	15
Types of subjects studied:	Not specified
State of subjects studied:	15 estimations at rest 11 estimations during exercise 8 estimations during infusion of isoproterenol hydrochloride infusion (2 μg./min.)
Dye used:	Indocyanine green
Injection site:	P.A.
Sampling site:	Brachial artery
Sampling method:	Continuous recording. Withdrawal via motorized constant-speed syringe
Number of dye curves per Fick:	1
Duration of oxygen uptake determination:	3 minutes, except for exercise collections of 1 - 2 minutes
Mixed venous blood samples:	P.A. samples taken throughout first and third minutes of oxygen uptake period, except during exercise where taken throughout each of two successive 30 second periods during oxygen uptake

Comments: Dye and Fick estimations not simultaneous.

THE CHOICE OF THE TYPE OF INDICATOR-DILUTION TECHNIQUE USED IN THIS STUDY, AND ITS JUSTIFICATION

The Requirements of the Method

Before going on to discuss the relative merits of the various components of the dye-dilution system used, it is appropriate to outline the case against the other methods of indicator dilution, the development of which was outlined in a previous section. Due consideration must be given to the various interrelated components of any system. Certain indicators may seem ideal, but detection devices are inadequate for their use, while the reverse may be true, and so the selection of any system must be a compromise between all the factors and components involved. A further aspect which must be taken into account is the main purpose for which the system is chosen, in this case rapidly repeatable and accurate measurement of cardiac output, while aiming at maximum versatility for the measurement of cardiac output under a variety of conditions, regional blood flow studies, diagnostic studies in intracardiac shunts, and useful by-products of the indicator-dilution method, such as circulation times and intravascular volumes.

Conductivity Methods

First, let us consider the original principle used by Stewart in 1897, the conductivity method. This offers the enormous advantage that the sensing device can be built into the catheter tip in the form of electrodes, thus overcoming the hydraulic distortion of the curve caused by traversal of a sampling system, as well as avoiding blood loss during

the procedure. The use of saline, Ringer's solution and similar electrolyte solutions however, has the great drawback in quantitative studies of inconstant loss by diffusion in the lungs, thus rendering the injection dose an indefinable quantity, when its accurate measurement is of vital importance in the calculation of cardiac output. In studies where a capillary bed is not interposed between the injection and sampling sites, this difficulty does not arise. Holt (1956) has pointed out the considerable margin of error in calibrating the conductivity method, as the conductivity cell is sensitive to changes in temperature, haematocrit and flowrate. Goodwin and Sapirstein (1957) showed also that, with the use of hypertonic saline as an indicator, the resistance of the blood is not predictably related to the indicator concentration, making an empirical calibration in vitro necessary, which may not necessarily reflect the in vivo situation. They therefore used autogenous plasma as an indicator, but required large injection volumes. This was overcome by the use of a solution iso-osmolar and iso-conductive with plasma, introduced by Hershgold et al. (1960). This, however, still required a large injection volume (7 - 15 ml. in dogs) to obtain adequate curves, despite greater amplification of the signal to reduce as far aspossible the injection volume. In addition, a "form factor" has to be determined for every new conductivity cell and for every withdrawal rate used. Use of this synthetic injectate, however, has overcome the loss of indicator in the lungs, which has bedevilled conductivity techniques in the past.

Thermal Dilution Methods

Thermal dilution techniques appear superficially a most attractive form of indicator-dilution method. Their instrumentation is technically simple, no blood withdrawal is required (always a great advantage in physiological studies), and they record the indicator-dilution curve at its site, undistorted by a sampling system. The indicator disappears rapidly from the circulation, and estimations can be performed repeatedly. Closer scrutiny, however, reveals certain drawbacks in the technique.

The problem of heat exchange between the blood-indicator mixture and its surroundings in transit from injection to detection site is the major problem. In large-bore, thick-walled vessels in poorly vascularized tissues with high blood velocities this exchange is slight; in thin-walled, small diameter vessels, with slower flows in highly vascular surroundings, exemplified by capillary beds, the greatest heat exchange will occur (Hosie, 1962). As Fegler (1953; 1954) so elegantly demonstrated in his model and subsequent animal experiments, the lungs are a specialised capillary bed with exceptional thermal insulation properties, and so minimise this heat exchange. Cardiac outputs performed by injection and sampling on opposite sides of the pulmonary circulation therefore do not suffer from this error to any great extent. Heat exchange and re-exchange, however, distorts the curve, rendering estimates of mean transit time and central blood volume impossible. Evonuk et al. (1961) have diminished the temperature gradient, which encourages heat exchange, by the use of room temperature injectate, and so reduced this error. The effect of

heat exchange between the intravascular portion of the catheter itself and the bloodstream, however, introduces errors, and can best be overcome by measuring the time course of the temperature of the injectate as it leaves the catheter with a thermocouple at its tip. Following injection, however, the contents of the intravascular section of the catheter will return gradually to blood temperature, which in effect amounts to a continuation of injection of "coldness", although at a very slow and progressively diminishing rate.

Because of this heat exchange problem, sampling and injection sites should be as near to each other as is compatible with adequate mixing. Conventional intravenous injection with sampling from a peripheral artery is therefore unsatisfactory. Detection must be as central as possible, preferably at the aortic root, to avoid heat exchange en route to a peripheral artery embedded in well-vascularised tissue. A guarantee of this therefore requires radiographic screening of the injection and detecting catheter with their mounted thermocouples, to achieve the desired position.

Froněk and Ganz (1960) have recently done some interesting work on regional flow by a single catheter technique, whereby cool saline or dextrose solution is injected upstream in a jet, rapid enough to cause local turbulence and adequate mixing, to allow registration of representative temperature by a thermistor mounted only a few millimetres downstream on the same catheter. This method obviously minimises heat exchange

problems, but, as found in the studies of Andres et al. (1954) and Grace et al. (1957), an injection velocity, sufficiently rapid to cause adequate mixing, probably causes haemolysis with attendant vasomotor changes due to the release of vasodilator substances. Moreover, the authors point out that high velocity injections, such as are necessary, are sufficient to accelerate considerably the flow being measured, and necessitates dividing the dilution curve into two parts, with solution of two simultaneous heat-balance equations. This, however, is not a problem peculiar to thermistor techniques, and could equally well apply to dye-dilution methods in the measurement of regional flow, with injection and sampling sites in such close proximity.

In general, it seems that for the measurement of cardiac output by thermal dilution techniques to the same degree of accuracy expected from other indicator-dilution methods, the precautions taken against heat exchange in most studies to date are sufficient. Nevertheless, a full awareness of the possible shortcomings of the method should be retained, and the method should not be applied without adequate controls to conditions different from those in which it has up until now shown successful application (Hosie, 1962).

Haemodilution Methods

Haemodilution techniques can be dismissed as a serious contender in the choice of indicator-dilution technique. Even in the most recent study by Lochner and dal Ri (1957), large volumes of indicator, whether saline, plasma or dextran, have to be injected to produce curves of adequate dimensions, and the problem of obtaining an absolute calibration has not been solved.

Gaseous Indicators

Gaseous indicators, radioactive or otherwise, are unsuitable for the measurement of cardiac output, although they have very definite value in detection of left to right and right to left shunts, and in organ blood flow studies. Since the measurement of cardiac output is the main requirement of the technique under discussion, they do not really compete, with the possible exception of Kr^{85} dissolved in saline, as used by Cournand's group (Chidsey et al, 1959; Rochester et al., quoted by Hamilton, 1962) for a continuous infusion method of measuring cardiac output. This however entails discontinuous sampling, which would surely be a retrograde step as a routine in repeated cardiac output estimations, especially with its attendant blood loss.

Radioactive Indicators including Radiocardiographic Methods

Radioactive indicator techniques are receiving increasing attention, and with recent improvements in instrumentation are becoming increasingly accurate. External counting methods have justifiably been explored, as they overcome the decided disadvantage of other indicator-dilution methods of arterial puncture. The necessity of repeated arterial puncture has discouraged serial studies on the same subject, which would otherwise have frequent application in following patients' courses in certain cardiovascular disorders, or in the careful assessment of the normal haemodynamic phenomena of pregnancy.

Radioactive indicator techniques can be divided into three types. Firstly, the method involving multiple sampling with individual analysis

of each sample for radioactivity, although accurate, it can be dismissed as a poor competitor. Whatever the method which has to compete with the other indicator-dilution methods, it must employ continuous recording. The second method involves withdrawal of blood via a counting device which gives a continuous record of radioactivity. Apart from the possibility of indicators such as Kr^{42} diffusing out to some extent in the pulmonary bed (Conn, 1955), and I^{131} albumin causing errors due to its tendency to stick to glassware (Crane et al., 1958), radioactive indicators have no advantage over dyes such as indocyanine green. Their severest drawback is the dose limitation which they impose upon the number of estimations which can be safely performed in any one patient. For example, only six to eight estimations of cardiac output using Kr^{42} can safely be done per week on a patient (Conn, 1955). The exigencies of the method limit the dynamic response characteristics of detection and counting instruments, making them less applicable in rapid circulations, and introducing unwanted distortion in the indicator-dilution traces. Apparatus is costly, and the use of radio-isotopes introduces radiation hazards into the laboratory, without bringing any particular advantages with them.

External counting techniques have, as Conn (1962) puts it, "an almost 'Lorelei-like' appeal". The only discomfort suffered by the patient is a venipuncture, large numbers of patients can be studied with negligible morbidity, the indicator can be used in trace amounts, and the detection equipment is highly sensitive. Because of the minimal trauma to the patient, the physiological state is not unduly disturbed,

and the recordings are not influenced by oxygen saturation or non-specific changes in the optical density of the blood. Chemical procedures are limited to the preparation of the indicator, and analysis of the data reaches the same stage of processing as do dye-dilution traces. It may seem strange that, with so many apparent advantages, the method has not achieved more widespread use. One of the major factors has been that not all comparisons with well-established cardiac output methods, such as the Fick or dye-dilution methods, have been completely convincing (Carter et al., 1959; Gorten and Gunnells, 1960; 1961), as mentioned earlier.

As in all indicator-dilution methods for cardiac output measurement, the detection instrument must be calibrated. With external counting methods, the calibration must be accomplished in vivo by a comparison of the praecordial counting rate with the absolute concentration of isotope in peripheral blood in microcuries per millilitre at a time following the initial injection when the isotope concentration in blood has reached an equilibrium value. This implies that all intravascular radioactivity contributing to the final counting rate during calibration should reside within the same circulatory segments contributing radioactivity during the earlier inscription of the dilution curve. The segments initially contributing activity are a variable combination of the heart chambers and great vessels, according to the positioning of the detector. When the peripheral venous sample is eventually taken for an absolute count value, the praecordial count is obviously contributed to also by the radioactivity in large intrathoracic venous segments, chest wall vessels,

and myocardial vessels. To the extent that these additional segments contribute, the calibration is invalid, the counting rate spuriously high, and the cardiac output overestimated. It follows naturally that the position of the detector in relation to its original field must not alter between recording cardiac output and the calibration count (Crane et al., 1960). The extent of the contaminating contribution varies with differences in the technical details of measurement, particularly with differences in positioning of the detector. Opinions differ whether technical performances of the method can be standardised to keep the unwanted contributions of radioactivity to a predictable level and as small as possible (Conn, 1962).

In order to count the radioactivity of blood passing through the heart, one must accept some contribution from isotope activity in blood of neighbouring tissues. In order to obtain counting rates sufficiently great to give statistically valid curves from the injection of a dose of isotope small enough to ensure safety, it is frequently necessary to minimise collimation, and allow the crystal to scan a relatively broad expanse of these tissues. Herein lies the greatest stumbling block in the successful measurement of cardiac output by external counting techniques.

A point in favour of the costly outlay for external counting equipment is its versatility. It is becoming increasingly used for detection of congenital cardiac shunts (Greenspan, Lester and Marvin, 1957; Greenspan, Lester, Marvin and Amplatz, 1959 a, 1959 b; Shapiro and Sharpe, 1959; Pritchard et al., 1959; Turner, Salazu and Gordon, quoted by Conn, 1962), although the accuracy obtainable is somewhat lower,

and rather less certain than with direct sampling indicator-dilution techniques. It has a very definite place, however, as a screening procedure, and in young children in whom arterial sampling may be difficult.

It has application in measurement of regional blood flow (Smith and Quimby, 1945; Kety, 1949; Dobson and Warner, 1957) where standard dye methods may not be practicable. Worthy of mention are its applications in the I^{131} diodrast renogram (Winter, 1956), the I^{131} rose bengal test (Taplin, Meredity and Kade, 1957) and the radiogold method for estimating liver blood flow (Burkle and Gliedman, 1959). These three techniques reflect blood flow, but are a complex function of blood flow, parenchymal uptake, metabolism and secretion; they nevertheless have definite value though they do not measure flow in absolute units.

In conclusion, therefore, external counting methods cannot be said to rival dye-dilution methods in the accurate measurement of cardiac output, although they are of value as screening tests, and have many additional applications in circulatory studies for which they can be adapted. In a search for an ideal method of measuring cardiac output accurately in varying situations as the paramount consideration however, neither they, nor any of the other methods discussed, are serious challengers.

METHODS

A DESCRIPTION OF THE METHOD OF DYE-DILUTION USED IN THIS STUDY FOR THE ESTIMATION OF CARDIAC OUTPUT

The following is a brief description of the injection, sampling, detection and recording system used for the inscription of dye-dilution curves in this study, and the method of calibration and calculating flow.

A solution of indocyanine green (Cardio-Green, Hynson, Westcott and Dunning, Inc.) of known concentration and constant volume was injected from an A.R.H. pipetting unit, which automatically reloaded itself from a dye reservoir flask within approximately two seconds. By manual depression of a lever attached to the plunger of the syringe, injection was performed by a process of displacement, via a dye-filled catheter. The exact time and duration of the injection was simultaneously inscribed by a square wave time marker, which deflected the baseline of the dye trace on the ultraviolet recorder.

Meanwhile, arterial blood was being withdrawn from the arterial system via the densitometer cuvette, and the baseline thus inscribed by the recorder. The arterial blood was sucked through a heparinized, saline-filled 4F Portex nylon catheter, of internal diameter 1.00 mm. and external diameter 1.34 mm. inserted into the subject's brachial artery. The catheter had a female luer connection which was attached over a male luer connection on the densitometer cuvette. The densitometer cuvette was a Waters XC-250A model, with a single optical system designed for peak response at the wavelength of maximal absorption of indocyanine green (800mμ). After traversal of the densitometer cuvette, the blood

passed through a length of nylon tubing of internal diameter 2.0 mm. and external diameter 3.0 mm., to a system of Hansen taps. It then travelled via a further length of the same nylon tubing to a 50 ml. Luer Lock syringe, mounted on a Harvard 600 - 900 single infusion-withdrawal pump which was modified to withdraw blood at an absolutely steady rate of 38.0 ml./min.

When the dye curve had reached a point well after the onset of recirculation, as seen on the oscilloscope trace, the recorder operator switched off the recorder, and the pump operator reversed the pump, thus returning the blood to the subject. Depending on the rapidity of the subject's circulation, and the frequency of the dye curves, the whole system, including the withdrawal syringe, taps, densitometer lumen and catheter was then flushed with heparinized saline. With rapidly consecutive dye curves it was sometimes not possible to flush the system until up to five consecutive curves had been inscribed.

A densitometer control unit supplied electrical power to the cuvette optical system, and controlled overall system operation, its output being fed into a mirror galvanometer (N.E.P. Type BB 30) of a N.E.P. twelve-channel ultraviolet recorder and an Airmec four-channel display oscilloscope (Type 279) incorporating a low speed time base (Type 318).

Before the start of any study, 100 ml. of blood were removed with dry syringes, and run into a siliconed flask with constant agitation of the blood. The flask contained only 1.5 ml. of 25,000 units/ml. heparin, and the blood was kept at body temperature. Provided the heparin and blood were adequately mixed this was quite sufficient to anti-coagulate

the blood, and any dilution errors were thus minimised by the small volume of anti-coagulant used. The same dye solution used during the study was added after the procedure to three 25 ml. aliquots of the blood, over a range calculated, on the basis of previous observations, to produce a maximum concentration deflection slightly higher than the peak concentration in the recorded curves, andthe remaining 25 ml. of blood was used for a baseline control. The volume of dye solution added never exceeded 0.188 ml., and was measured to the nearest 0.001 ml. In a 25 ml. sample, therefore, a volume of 0.188 ml. caused a dilution effect of only 1 in 133.

The concentrations of the calibration specimens made up for studies of cardiac output with central injection and sampling, with an injection weight, varying between subjects, of 1.548 - 3.986 mg. (average 2.624 mg.), and recording at a medium-high sensitivity, were usually 0, 2.5, 5.0 and 7.5 mg./l. At the end of the study, the calibration blood samples were drawn through the cuvette at the same suction rate as that at which the dye curves were inscribed, and at a slower recorder paper speed, and a trace such as that shown in figure 3 was obtained.

Finally, four aliquots of the dye solution used in the study were injected from the assembled dye injection apparatus through the injection catheter used into four dry weighed flasks. These flasks were reweighed, and the average of the weight differences in grams represented the volume per injection in millilitres. Injection volumes averaged 1.450 ml. (range: 0.955 - 1.569 ml.) and in any one study the four estimations were

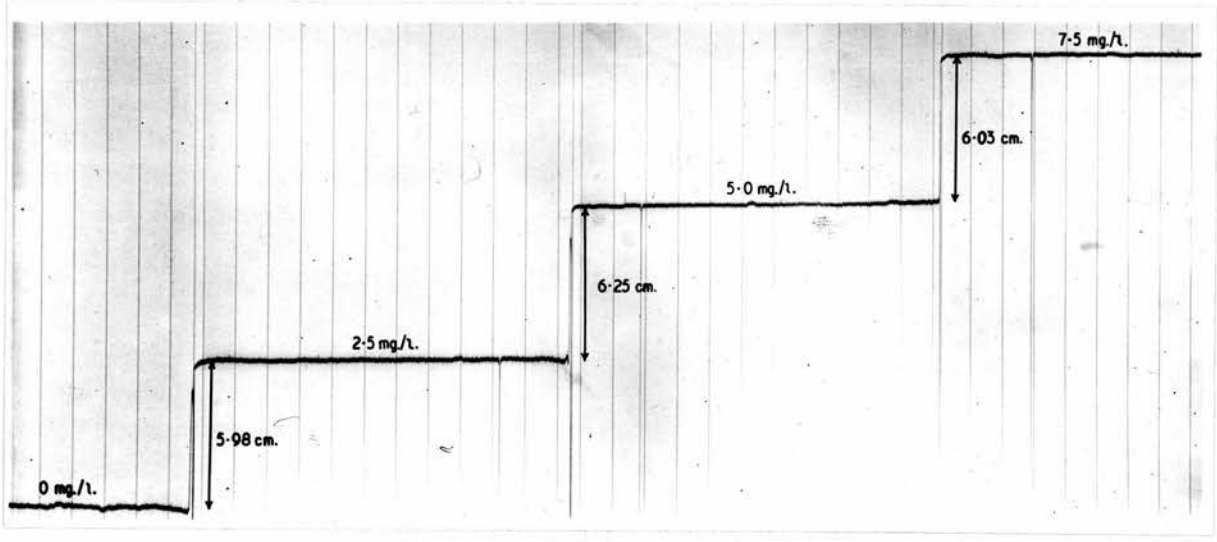

Figure 3: Calibration at sensitivity nine of dye concentrations 0, 2.5, 5.0, and 7.5 mg./l. in blood

required to agree within 0.01 ml.

Cardiac output in l./min. was calculated from the formula:

$$Q = \frac{60\ I}{\int_0^\infty c(t)\ dt}$$

as defined in a previous section on the theory of indicator-dilution methods. Since the same dye solution was used in the injection assembly and for the subsequent calibration, an accurate knowledge of its concentration was not necessary, and the manufacturer's stated weight in milligrams and the volume of diluent, to the nearest millilitre, were used to calculate its concentration. The volume of each injection was calculated as described above, and the value of I could therefore be determined in milligrams by multiplying the volume and the concentration of the injectate.

The tracing of the dye curve was allowed to develop slowly in a subdued light, and measured in a room shielded from natural sunlight. The baseline was prolonged under the curve itself. Unduly pulsatile traces were meaned freehand. Readings to the nearest tenth of a millimetre were taken at one-second intervals with a set square laid along the baseline of the dye curve. The presence of vertical time marker lines at intervals of one-tenth, one half-second or one-second intervals on the trace facilitated this process. These readings were replotted on semilogarithmic paper, and where recirculation became apparent, as discussed earlier, the downslope was extrapolated. Readings taken from the replotted curve to a lowest reading of one millimetre on the extrapolated portion were then summed. A calibration factor was obtained by plotting the deflections in centimetres produced by the known concentrations of dye, usually 0, 2.5,

5.0 and 7.5 mg./l., against these concentrations. This factor, which converted centimetres into milligrams of dye per litre of blood, was then multiplied by the value of the sum obtained above, to give the value for $\int_0^\infty c(t)\,dt$, and cardiac output was thus calculated.

DISCUSSION OF THE COMPONENTS OF THE SYSTEM USED

Having decided to use a dye for the indicator study, it follows that the various components of the system, as described above, should be justified individually and in relation to one another.

The Choice of Dye

Before going on to describe the properties and merits of indocyanine green used in this study, it is appropriate to discuss some of the other dyes available for such a study and their individual shortcomings, bearing in mind the properties of an ideal dye:

i) The dye must be water-soluble and suitable for sterile intravenous injection in a small volume.

ii) It must have no injurious effect on the organism when administered in doses suitable for the determination of its concentration, and must in no way cause any physiological disturbance.

iii) Its spectral transmission must stabilize rapidly in blood, and it must be easily and accurately identified and measured in blood.

iv) The dye must not be lost from the blood in transit from the site of injection to the site of detection, or in

any way metabolically degraded into undetectable forms during this time.

v) It is desirable that the dye should be excreted within a reasonable space of time, preferably completely, after its first circulation, although this vitiates its use for subsequent determination of total blood volume.

vi) Its detection should not be interfered with by normally occurring physiological variations in oxygen saturation of the blood.

vii) It should have a high spectral absorption.

The Red Dyes: With the advent of the visual colorimeter brilliant vital red was the first dye to be used in the measurement of cardiac output (Hamilton et al., 1931). With a spectral absorption peak of 507mμ in plasma (Gregersen and Gibson, 1937), its quantitative recording is interfered with by the presence of haemoglobin, and its use is therefore restricted to discontinuous sampling methods only, with analysis of individual plasma samples. Its optical density is slightly affected by variations in protein concentration (Gregersen and Gibson, 1937), and plasma samples must be free from any haemolysis, and may require dilution with alcohol to eliminate fatty turbidity and adventitious protein-bound colour (Dow and Pickering, 1950). The dye shows considerable variation in its purity, even from the same manufacturers (Gregersen and Gibson, 1937). It attaches itself to plasma albumin and remains intravascular for some

time, giving the subject's skin a pinkish tinge (Hamilton, 1962).

Congo Red has a spectral absorption peak of 506mμ, and the same problem of intermittent sampling and tedious plasma sample analysis therefore applies, as with brilliant vital red. Although its spectral absorption peak is very near an isosbestic point for reduced and oxy-haemoglobin (505mμ), as with brilliant vital red, continuous recording techniques would not be feasible due to the extreme opacity of blood in this region of the spectrum.

Rose Bengal has its spectral absorption peak at 560mμ. It stains the stools red, and has a peculiar photodynamic action which causes haemolysis of red blood cells on exposure to sunlight. Patients should therefore be kept in a darkened room for a variable period after its administration (Kerr, Delprat, Epstein and Dunievitz, 1925). Brod, however, claims to have used Rose Bengal in some thirty patients in a total dose of up to 400 mg. per patient, given over three to five hours, without any untoward effect (Brod, personal communication). He points out, however, that it is very difficult to obtain Rose Bengal supplies suitable for human use.

Indigo carmine is a vat dye with a rather variable composition therefore. Its spectral absorption is rather like that of Evans blue, with a maximal absorption in the range 600 - 610mμ (Lacy, Ugaz and Newman, 1955), and, like Evans blue, its recording in whole blood is interfered with by fluctuations in haemoglobin oxygen saturation. When it was introduced in dye-dilution studies in 1955 (Lacy et al., 1955) it had

the great advantage over the then-popular Evans blue of less stringent dosage limitations, as its removal from the body is far quicker than that of Evans blue, thus avoiding discolouration of the patient. It is, however, a much weaker absorber than Evans blue, requiring doses three to four times greater. This factor, combined with its relative insolubility in water, necessitates injection volumes far larger than are desirable in dye-dilution studies.

All studies reported to date have used discontinuous sampling techniques, with the exception of one by Wood's group, quoted by Fox (1962), where continuous recording with indigo carmine was successfully employed (Birkhead, Fox and Wood, unpublished data). Unlike the other red dyes, therefore, it seems that continuous recording techniques can be used with indigo carmine, presumably because its spectral absorption peak is the highest of them all. Blood is fairly opaque even at this wavelength however, and the dosage required must be even larger than those necessary for discontinuous sampling methods.

The Blue Dyes: Methylene blue is photometrically superior to Evans blue as it absorbs more light at the peak sensitivity of the red photocell-filter assembly used in oximetry at 640mμ, although in fact its molecular content is three times that of Evans blue for the same concentration. Its great advantage is that it does not stain the skin like Evans blue due to a rapid decrease in spectral absorption, by movement of the dye into the cells and intracellular reduction to the colourless leuko form (Fox and Wood, 1957 a). This has led to its use in diagnostic studies of congenital heart disease where dose limitation with Evans blue has

presented a problem. This advantage of methylene blue, however, proves also to be its greatest drawback, as quantitative spectrophotometric estimations cannot be made in the measurement of cardiac output. Like Evans blue, its recording is interfered with by fluctuations in haemoglobin oxygen saturation.

Coomassie blue, the most recent of the blue dyes for use in cardiac output estimation, was introduced in 1959 (Taylor and Shillingford, 1959; Taylor and Thorp, 1959). It offered an advantage over Evans blue in that it was rapidly cleared from the circulation, thus overcoming the cosmetic objection of Evans blue (Connolly and Wood, 1954; Taylor and Thorp, 1959). Unfortunately however, large doses of as much as 30 mg. are necessary to obtain adequate sized curves. Hoffman and Guz (1961) have reported toxic reactions to doses of 798 mg. given over 54 minutes, and Resnekor (1962) has reported similar reactions with doses over 500 mg. by serial injections, although Taylor and Shillingford (1959) claim to have given as much as 1000 mg. intravenously in a single injection without ill effect. Coomassie blue also has the disadvantage of being interfered with by variations in oxygen saturation.

Evans blue (T-1824) merits rather fuller discussion that the aforementioned dyes since, until the advent of indocyanine green, it was undoubtedly the most widely used of all the dyes for cardiac output estimations. The development of devices for recording dye curves continuously in blood after the 1950's was almost entirely geared to the detection of Evans blue. Moreover, it was widely used for plasma volume

estimations because of its very slow removal from the circulation where it is bound to plasma albumin (Connolly and Wood, 1954). This advantage of obtaining total plasma volume has been lost with the use of indocyanine green, but it is more than outweighed by the many drawbacks of Evans blue.

One of the greatest of these is its dose limitation. The sole reason for this is cosmetic, for in doses greater than approximately 0.5 mg./kg. it stains the skin an unhealthy bluish tint which takes several weeks to fade (Connolly and Wood, 1954; Taylor and Thorp, 1954). Since an average of five milligrams is needed for injection for each dye curve in an average patient, this imposes a limit of approximately seven curves per patient. This has been one of the main points in favour of other blue dyes such as methylene blue and coomassie blue which leave the circulation more rapidly.

The second major drawback has been that Evans blue could be used with accuracy only in the arterial circulation in patients breathing oxygen. Because its spectral absorption peak lies in the visible region of the spectrum where reduced haemoglobin also has its maximal spectral absorption, reduced haemoglobin interferes with its detection and measurement, and conversely Evans blue interferes with oximetry. Where right to left shunts are present even breathing 100 per cent. oxygen may fail, due to the fluctuating oxygen saturation of arterial blood caused by alterations in the volume of the veno-arterial shunt with the respiratory cycle, and such curves may be uninterpretable (Swan and Wood, 1953). Use of Evans blue obviously precludes any accurate continuous recording dye studies sampling

from the venous circulation where oxygen saturations fluctuate even in normals (Wood, Bowers, Shepherd and Fox, 1955). Fully appreciating this limitation, Wood's group at the Mayo clinic used a two-colour oximeter, which provided a compensatory infrared measurement to compensate for interference by non-specific optical density changes such as the reorientation of erythrocytes with the cardiac cycle, changes in haematocrit, etc. Their instrument was therefore able to inscribe curves free from interference of any sort from the arterial circulation in normals breathing 100 per cent. oxygen, and added considerably to the accuracy of their measurement. Until Sutterer's recent description of his dichromatic densitometer (Sutterer, 1960), this infrared compensatory wavelength was sacrificed in the monochromatic densitometric use of indocyanine green.

Although Evans blue molecules combine almost instantaneously with plasma albumin (Andres et al., 1954), the dye-blood mixture takes approximately two to eight seconds to stabilise due to an early overshoot in the spectral transmission of the mixture (Fox and Wood, 1960 a). Wherever the time between injection and sampling is short in continuous recording dye-dilution studies, this delay in stabilisation of the spectral transmission of the dye-blood mixture will interfere with the quantitative determination of Evans blue concentration.

A final drawback of Evans blue is that appreciable amounts of rapidly metabolised impurities occur in standard solutions of Evans blue, which may affect quantitative estimations in vivo (Cooley, 1954).

The Green Dyes: About the same time as the introduction of indocyanine green, RIE 1743 was first introduced in Germany. It belongs to the same general group of dyes as indocyanine green, but is not suitable for indicator-dilution work because it forms aggregates in solution (Kramer and Ziegenrücker, 1957). Like indocyanine green, its detection is unaffected by the oxygen saturation of the haemoglobin in the blood.

Due to the combined efforts of Drs. Brooker and Heseltine of the Eastman Kodak Research Laboratories, and Dr. Fox of the Mayo Clinic, indocyanine green, a tricarbocyanine dye of molecular weight 775 and structural formula shown in figure 4, was developed, specifically to overcome the interference of oxygen saturation with continuous recording of dye-dilution curves (Fox, Brooker, Heseltine and Wood, 1956; Fox et al., 1957). Its advent constituted a major breakthrough in indicator-dilution techniques, and has overcome most of the drawbacks of previous dyes used for this purpose.

Firstly, what are its disadvantages? They are few, and can be dismissed briefly. The dye is unstable in aqueous solution, but not to any great extent. Fox and Wood (1960) have shown that the degree of deterioration is slow enough to permit its use for one to two days after making up an aqueous solution, and in its powdered form before solution it will keep indefinitely.

A second drawback is its expense. A 50 mg. ampoule and its solvent, enough in the system used in this study to inscribe approximately 20 dye-dilution curves for determination of cardiac output, costs £2. 18. 4d. in Britain.

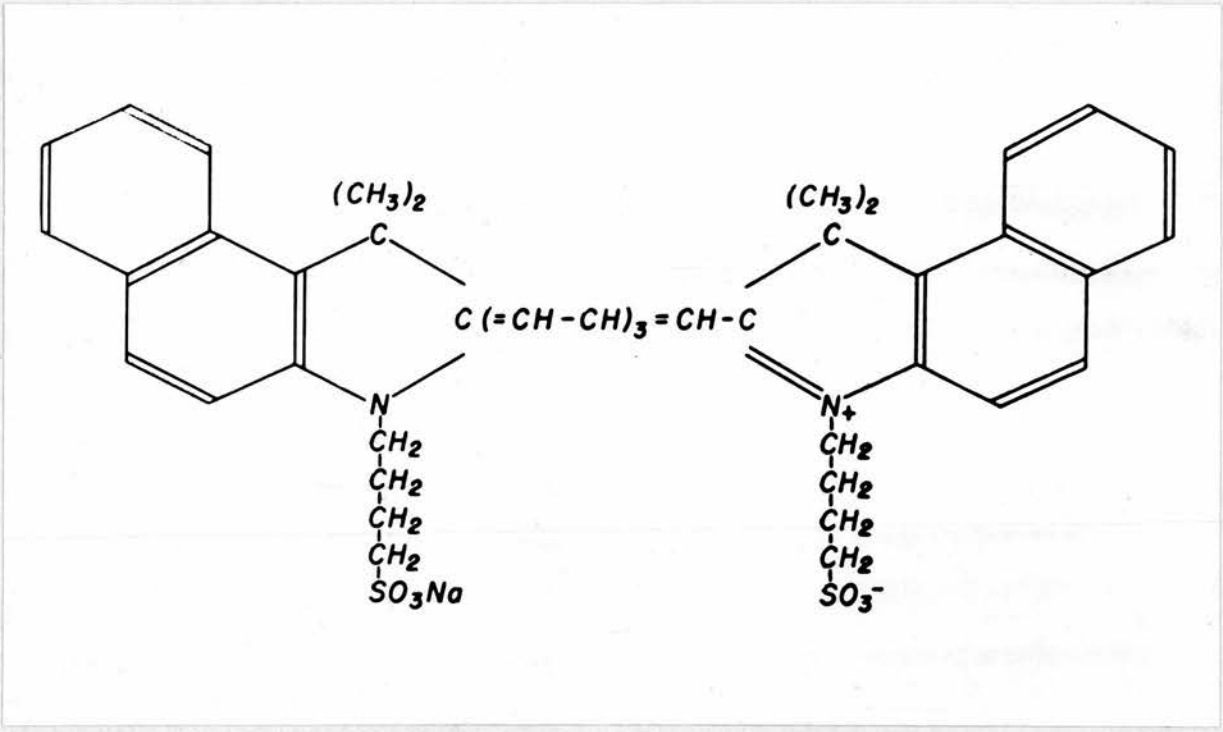

Figure 4: Structural formula of indocyanine green (Fox and Wood, 1960a)

A questionable disadvantage is that its rapid removal by the liver, with a very short half-life, means that it is quite unsuitable for total plasma volume estimations, unlike Evans blue. This however is inevitable, and is a small price to pay for its many other advantages over Evans blue.

Its final disadvantage cannot really be claimed to be a shortcoming of the dye itself. With the use of monochromatic densitometry, the compensatory effect of the other photocell in preventing interference by non-specific changes in optical density in blood, as was achieved by the converted oximeters, was lost. This, however, was the fault of instrumentation not keeping pace with development, and has now been overcome by the recent introduction of a dichromatic densitometer (Sutterer, 1960).

What of its advantages? Indocyanine green has its peak absorption at about 800mμ (figure 5), a so-called isosbestic point where oxyhaemoglobin and reduced haemoglobin transmit light equally (Fox et al., 1957). Transmission of light of this wavelength will therefore be affected by the total amount of haemoglobin in the blood, but will be independent of the proportions of oxyhaemoglobin and reduced haemoglobin present. Immediately, the tremendous advantages of this become apparent. Patients need no longer breathe 100 per cent. oxygen during the inscription of dye curves, curves can be recorded quantitatively from the venous circulation, the scope of diagnostic dye-dilution studies is enormously increased by detection on the right side of the heart, and organ blood flow can be investigated by sampling from the venous outflow of the organ. Bassingthwaighte, Edwards

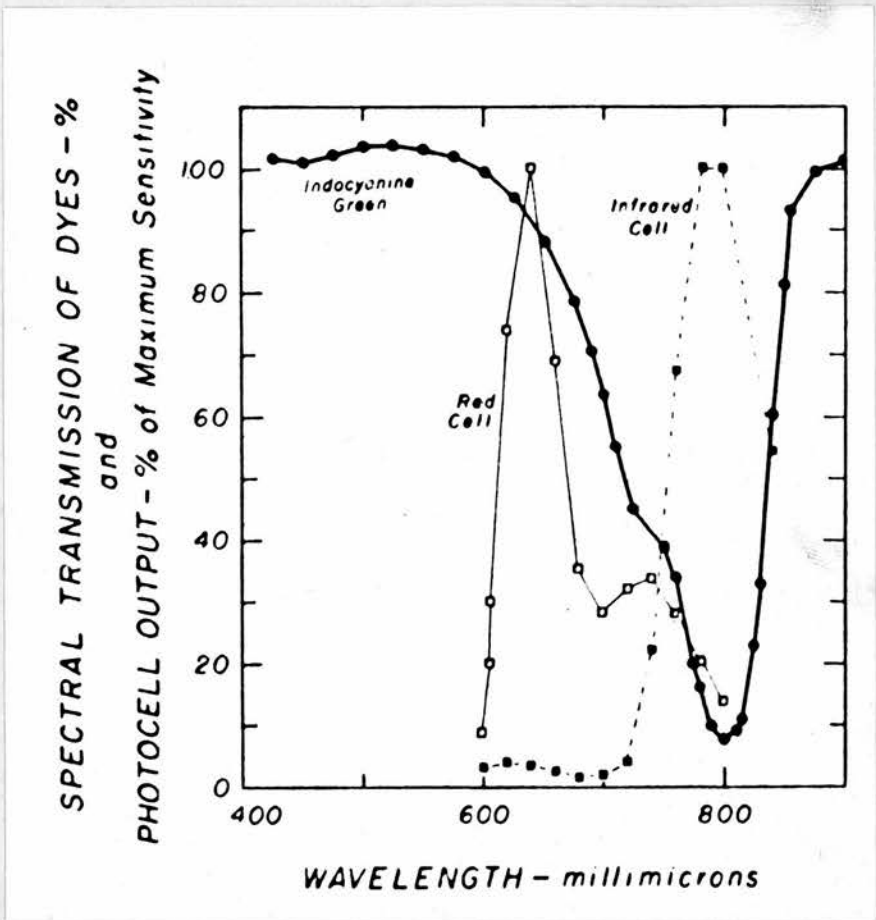

Figure 5: Light transmission of indocyanine green in plasma, and spectral sensitivities of red and infrared oximeter photocells (Fox and Wood, 1960a)

and Wood (1962) have recently suggested that the presence of reduced haemoglobin does affect the calibration of indocyanine green in blood, but not to any great extent. They showed that at the saturation of mixed venous blood fixed indocyanine green concentrations gave readings two to four per cent. lower than in arterial blood, and five per cent. less in coronary sinus blood with an oxygen saturation of approximately 20 per cent. This, however, was probably not a shortcoming of the dye, but of their instrument (Waters XC - 100A). It seems likely that the light obtained from their filter arrangement was not purely monochromatic, and was not therefore "tuned" absolutely to 800 mμ, thus giving insufficient immunity to changes in oxygen saturation.

As was mentioned in the discussion of Evans blue, it is essential that a dye-blood mixture should achieve a stable spectral absorption as soon as possible after they meet. If the coefficient of light absorption continued to increase after analysis began at a central sampling site, it would be difficult or impossible to make quantitative measurements of the concentration of indicator from such a curve. Therefore the time required after the dye first meets the blood for the development of a stable coefficient of light absorption at the wavelength at which the densitometer is most sensitive to the indocyanine green-blood mixture, is of critical importance. Indocyanine green forms a complex with plasma proteins, changing its spectral absorption, and shifting the wavelength of peak absorption, a physicochemical process of finite duration. Fortunately, however, it has been convincingly shown that this occurs

within one to two seconds of its injection into the blood (Fox and Wood, 1960; Sinclair et al., 1960; Bassingthwaighte et al., 1962).

A most reassuring quality of indocyanine green is its freedom from toxic reactions. In this laboratory, doses as high as 3 - 4 mg./kg. have been given over a period of four hours with no side-effects, and in none of the patients studied have there been any ill-effects from its administration. The literature as well contains no reports of any adverse reactions from its use. Merriman et al. (1958) checked haemoglobin concentration, packed cell volume, total white cell count, differential white cell count, thymol flocculation, thymol turbidity, zinc sulphate flocculation, albumin-globulin ratios including electrophoresis, urinary specific gravity, sugar, acetone, protein and microscopy, before and after giving indocyanine green, and demonstrated no ill-effects.

Indocyanine green is rapidly and exclusively removed by the liver, and excreted more slowly in the bile (Wheeler, Cranston and Meltzer, 1958; Cherrick, Stein, Leevy and Davidson, 1960), which means that its use in regional blood flows is not in any way interfered with by diffusion or extraction losses, except across the liver. Its half-life has been shown by various workers to be between 5 - 11 minutes (Fox, Brooker, Heseltine and Essex, 1957; Wheeler et al., 1958; Rapaport, Ketterer and Wiegand, 1959; Ketterer, Wiegand and Rapaport, 1960).

An interesting and serendipitous discovery has been that this dye, originally developed for use in cardiac output studies, because of its exclusive removal by the liver, is ideally suited to the estimation of

hepatic blood flow, and this attribute is being fully exploited (Murray and Nebel, 1959; Rapaport et al., 1959; Ketterer et al., 1960; Reemstsma, Hottinger, de Graff and Creech, 1960; Caesar, Sheldon, Chiandussi, Guevara and Sherlock, 1961).

The optical density of indocyanine green is unaffected by variations in plasma pH from 7.0 - 7.8, variations in plasma protein concentration from 10 - 100 per cent., and in sodium chloride concentrations from 0.3 - 1.5 g./100 ml. of plasma (Fox and Wood, 1960). In work done at the beginning of this study of comparing dye-dilution and Fick estimates of cardiac output, the presence of indocyanine green was shown to introduce no errors in determinations of haemoglobin oxygen saturation by the Brinkman haemoreflector used in the dye-Fick comparison.

The Choice of the Detection Instrument

Photometry on whole blood incurs particularly difficult problems. Whole blood is a heterogeneous fluid of great density, and it is therefore necessary to examine very thin layers of blood, if sufficient light to produce a registrable photo-current is to be transmitted. The thinner the layer of blood, however, the smaller the changes in light transmission will be for a given amount of dye. This necessitates amplification of the photo-current for recording of dye dilution curves, but this in turn increases the effect of interfering changes in optical density, caused by factors other than the dye, such as haematocrit changes, and changes in red cell shape and orientation. The background optical density of flowing blood, due largely to the scattering and reflection of incident light by

the red cells, is constantly changing, due to changes in flowrate, haematocrit, carbon dioxide tension, etc. (Sinclair et al., 1961). In detecting dye-dilution curves therefore, one is measuring them against a background optical density of whole blood, which represents the major portion of the total optical density of the dye-blood mixture, and which is itself capable of changing.

Herein lay the great advantage of the two-colour oximeter cuvette, developed originally for estimations of oxygen saturation by the Mayo group. The Evans blue concentration was measured at 640 mμ, while the infrared photocell corrected for the variations in background optical density in flowing blood at the isosbestic point (800 mμ), where absolute haemoglobin concentration could be registered irrespective of its oxygen saturation. One of the shortcomings of the photomultiplier tube densitometers, discussed earlier, was that they were strictly one-colour instruments, until Falholt and Kaiser's somewhat disappointing attempt to introduce a compensatory band in the green range (Falholt and Kaiser, 1955). Gilford's instrument had poor dynamic response characteristics compared to the unmodified Wood oximeter, since its volume was 0.30 ml. as against 0.02 ml. of the volume overlying the detector photocell of the oximeter. Fox (1962) points out, however, that the addition of a zero-suppression circuit to compensate for background dye, and the practically linear relation between optical density and electrical output over the range of operation of the Gilford instrument were distinctly advantageous features.

The decision to use indocyanine green, and the necessity for a very rapid and linear response precluded the use of these instruments designed to operate with Evans blue as an indicator. Initially Wood's group modified their oximeter circuit, allowing their instrument to be used as a densitometer for the detection of indocyanine green with the infrared cell as the detecting element (Sutterer, Isaacson and Wood, unpublished data). Subsequently an improved densitometer, the Waters XC-100A, was developed, having more rapid dynamic response characteristics, with a photodiode as the sensing element (Edwards, Isaacson, Sutterer, Bassingthwaighte and Wood, unpublished data). To maintain constant sensitivity, despite rising background dye levels in the blood after repeated injections, certain modifications were necessary. A negative bias or zero-suppression circuit was incorporated in the circuit on the phototube side of the sensitivity control for the densitometer, and was adjusted so that the galvanometer reading with zero light on the phototube was in a negative position from its mechanical zero or zero-current position. This meant that the deflection measured from zero light increased to provide the same "blank" reading in the presence of background dye, and the relationship between galvanometer deflection and changes in dye concentration closely approached that predicted by Beer's law (Edwards, Bassingthwaighte, Sutterer and Wood, 1960). As will be described, the Waters X-250A model, used in this study, employs a far simpler method of coping with rising background dye levels.

The Gilford instrument, marketed as the Colson 103 IR densitometer,

has been modified by the use of a special infrared phototube, and the insertion of an appropriate interference filter, to give maximal response for indocyanine green at a wavelength of 805 mμ. It is constructed to allow presetting for suppression of background optical density of whole blood, so that any recorded change in optical density is due to dye in blood. Even if the densitometer is preset for suppression of increased background dye, there may be loss of sensitivity to increments of dye added to whole blood already containing dye (Miller et al., 1962). Like its predecessor, the instrument is expensive, bulky, has an unduly large cuvette lumen of 0.30 ml., and has the disadvantagesof the high tension supply system associated with photomultiplier photo cells, and the high gain amplifiers necessary to produce adequate signal outputs.

There are two British instruments commercially available for continuous recording of dye-dilution curves. The New Electronic Products instrument is a barrier layer cell assembly, suitable for monochromatic recording of dyes with spectral absorption peaks in the red and infrared range. It has been designed to produce a large output from the photocells by means of a strong light source. This causes heating of the photocells, which in turn necessitates cooling by the circulation of a stream of air through the lamp housing, lens, blood cell and filter faces to maintain thermal equilibrium. To reduce arterial pulsation artefacts, there is a relatively large volume, elastic catheter system between instrument and arterial lumen, which results in very poor volume-flow characteristics (Norman, 1959 a).

The Cambridge Mark II instrument is similar in design, and has similar volume problems, but does not require air cooling. It provides a compensatory infrared-sensitive cell to cancel out non-specific optical density changes when operating in the red range with blue dyes, as in Wood's instrument. Its infrared cell however, does not provide sufficient sensitivity with a high load resistance, and the output using indocyanine green is therefore alinear.

Before going on to a description of the Waters X-250A densitometer and its modifications, as employed in this study, the earpiece recording of dye curves should be mentioned briefly. Earpieces overcome the need for arterial puncture, which is a tremendous advantage. Unfortunately, however, they also have many disadvantages.

Firstly, they require a twenty minute warm-up period, during which time the ear must be treated to produce maximal vasodilatation, with either an infrared lamp or a histamine-containing cream. Due to the low intensity of light passing through the ear the electrical output is small, and extremely high amplification is necessary to record the optical density changes produced by the dye. As a result, slight movements between ear and earpiece can cause instability of the baseline, and drift.

Calibration of the ear oximeter is an inaccurate procedure, requiring venipuncture with direct spectrophotometric analysis of a spun plasma sample. Between the recording of the curve and the recording of the calibration deflection simultaneous with the blood sampling, the circulation of the ear must stay constant. The deflection measured is small in

comparison to the dye-dilution curve itself, and any baseline drift, between recording of the curve and that of the calibration deflection, will introduce considerable errors (Beard et al., 1950; Beard and Wood, 1951; Milnor et al., 1953; Warner and Wood, 1953; Prec and Cassels, 1955).

Moreover, against a progressively increasing level of dye in the blood during frequent injections in the same subject, some loss of sensitivity for each successive injection ensues (Norman, 1960).

The dynamic response characteristics are very poor, introducing many difficulties in the interpretation of dye curves in patients with rapid circulations (Wood et al., 1957). In infants an inadequate arterial supply to the ear may prevent its use altogether (Gabe and Shillingford, 1961).

The ear oximeter circuit has been modified for use with indocyanine green as a one-colour instrument (Sutterer, Isaacson and Wood, unpublished data), but uncontrolled changes in the blood content of the transilluminated ear cause indocyanine green curves to be less satisfactory than those recorded with the older blue dyes by conventional two-colour oximetry (Gabe and Shillingford, 1961; Fox, 1962). This, however, can be overcome to some extent by decreasing the instrument sensitivity and using much larger doses of dye, causing the variations in light transmission by the ear due to blood content changes to become insignificant in relation to the very much larger alterations caused by the dye (Fox, 1962).

The Modified Waters X-25OA Densitometer

The Waters X-25OA instrument consists of a single optical system designed for peak response at the wavelength where light absorption of indocyanine green is maximal (800 mμ). The densitometer cuvette optical system includes a light source, filter, lumen (sample chamber) and cadmium-selenide photocell. The densitometer control unit supplies electrical power to the cuvette optical system, and controls overall system operation.

Detection of indocyanine green is accomplished as follows. As the dye-blood mixture flows through the lumen in the cuvette between the light source and photocell, the amount of light striking the photocell changes its resistance in direct relation to the dye density in the optical path. This change unbalances a Wheatstone-type bridge circuit in the control unit, which provides a signal output proportional to the change in light intensity at the photocell, and a direct representation of the dye density. The photocell is sufficiently sensitive, and the filter so effective, that minimum dye dosage is possible, while still producing a signal output level of up to 100 mV., which does not require any amplification, unlike the Colson densitometer which requires high gain amplifiers.

The standard circuit was modified, however, to allow the output signal to be fed into both an ultraviolet recorder galvanometer and a high impedance oscilloscope (figure 6). This permits visual monitoring of the dye curve by the dye pump operator at the patient's side, which is a great advantage, as he can then direct the recorder operator without leaving the patient.

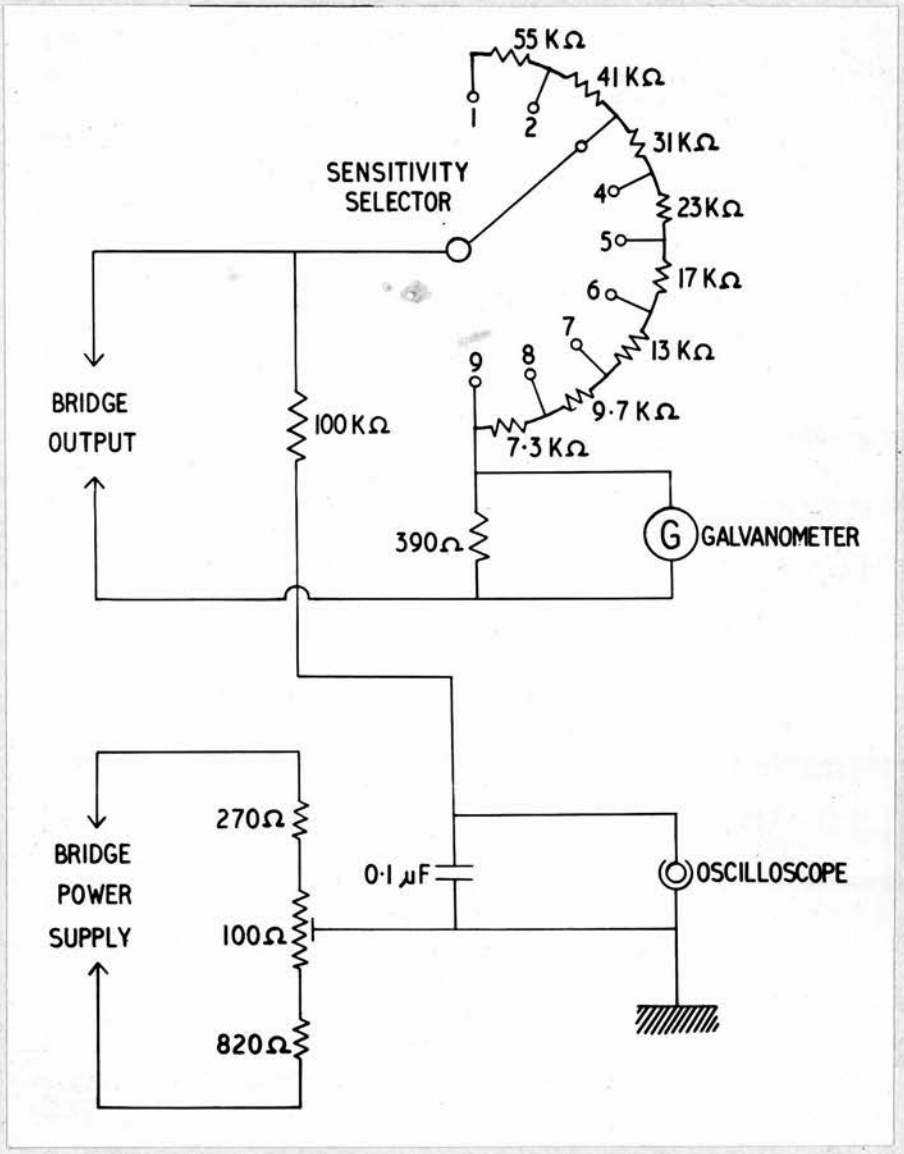

Figure 6: Diagram of circuit alterations

A further modification which has proved of great practical value is the substitution of a nine-position switch with a series of fixed resistors as a sensitivity control in place of the variable resistor originally fitted between the photocell bridge circuit and the recorder. The same photocell output can therefore give a variety of predictable galvanometer deflections in a ratio shown in Table 1, assuming the output value at sensitivity nine to be unity.

TABLE 1

Sensitivity	Galvanometer Deflection
9	1.00
8	0.75
7	0.56
6	0.42
5	0.32
4	0.24
3	0.18
2	0.13
1	0.10

The circuit arrangement is illustrated in figure 6. The value of this modification may not be immediately apparent. Patients have widely differing central blood volumes between injection and sampling catheters, which cannot be accurately predicted. An adequate sized dye injection for one patient may not give a curve of satisfactory height in another patient

using the same sensitivity if he has a larger central blood volume, since its peak concentration is a function of its dilution volume. If the dye-injection weight is predicted to give a suitable deflection at sensitivity six or seven, and it is found to produce a curve slightly too large or too small, a simple turn of the sensitivity adjustment can rectify the error, while still operating within the linear range of the system.

On the same principle, the same injection of dye can be used in the general circulation for cardiac output, and in a smaller volume of an organ circulation such as the kidney by adjusting the sensitivity, provided that the peak concentration attained in the circulation with the smaller volume remains within the linear range of the system. If then, one wishes to switch from regional flow to cardiac output and back again, one can always be assured of achieving identical sensitivities each time, and during subsequent calibration. A single calibration at the end of the investigation can then be applied to both the cardiac output and the regional flow calculations by conversion of the calibration factor derived at one sensitivity to the other, using the fixed and predetermined ratios quoted above in Table 1.

The problem always arises where repeated dye curves are performed in the same patient of rising background levels of dye in the blood, and its effect on instrumental linearity. Fortunately, the liver removes indocyanine green rapidly (Wheeler et al., 1958; Cherrick et al., 1961), but a steady increase in background dye levels can nevertheless occur where injections are performed at frequent intervals. In the Waters

XC-100A model this difficulty was overcome by the introduction of a zero-suppression circuit, as discussed earlier. In certain other instruments, such as the N.E.P. cuvette, compensation for background dye is achieved by adjustment of the bridge circuit, the balance of which is affected by the photocell output (figure 7). This procedure, however, shifts the photocell output onto a more alinear section of its response curve, introducing a progressively greater error as adjustments are made for increasing background dye.

In the X-250A model circuit however, the increasing background dye concentration is compensated for by increasing the light source intensity (figure 7), so that the photocell continues to work over the same range of its response curve which was chosen for its linearity. Photoconductive cells, in general, are noted for the linear relationship between their conductance and incident light at low levels, but a slight reduction in sensitivity was apparent in the present instrument as background dye reached significant concentration. Figure 8 illustrates the effect on linearity of raising the background level of dye from 0 - 12.5 mg./l. To assess the error which might have resulted from this slight effect of background dye, dye levels in the blood at the end of various procedures were estimated. In studies in which 50 or more dye injections of similar weight to those used in the dye-Fick study were given over approximately an hour and a half, dye levels in circulating blood never exceeded four milligrams per litre. In studies where between 25 - 50 injections were

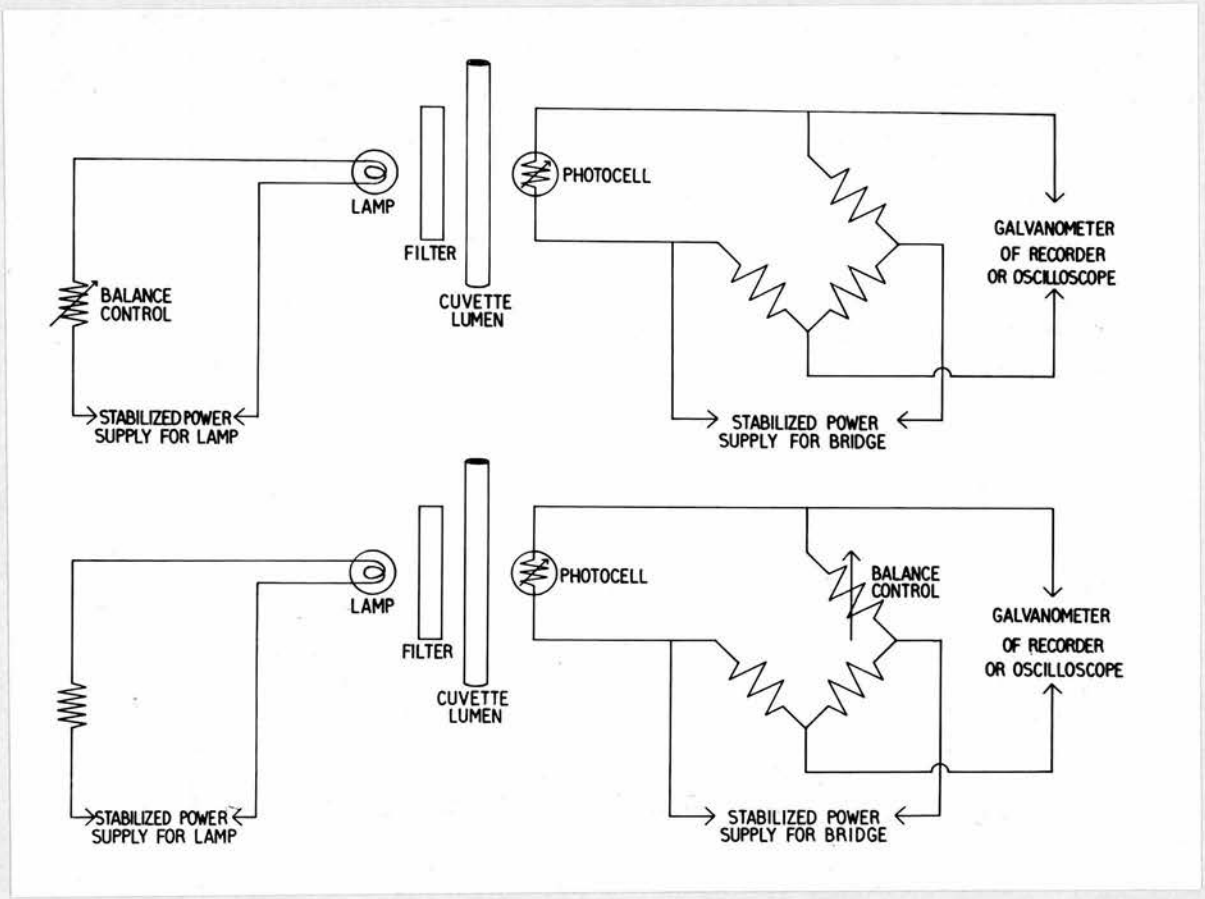

Figure 7: Circuit arrangements for compensating for rising background dye levels in the Waters instrument X-25QA (above) and certain other instruments (below)

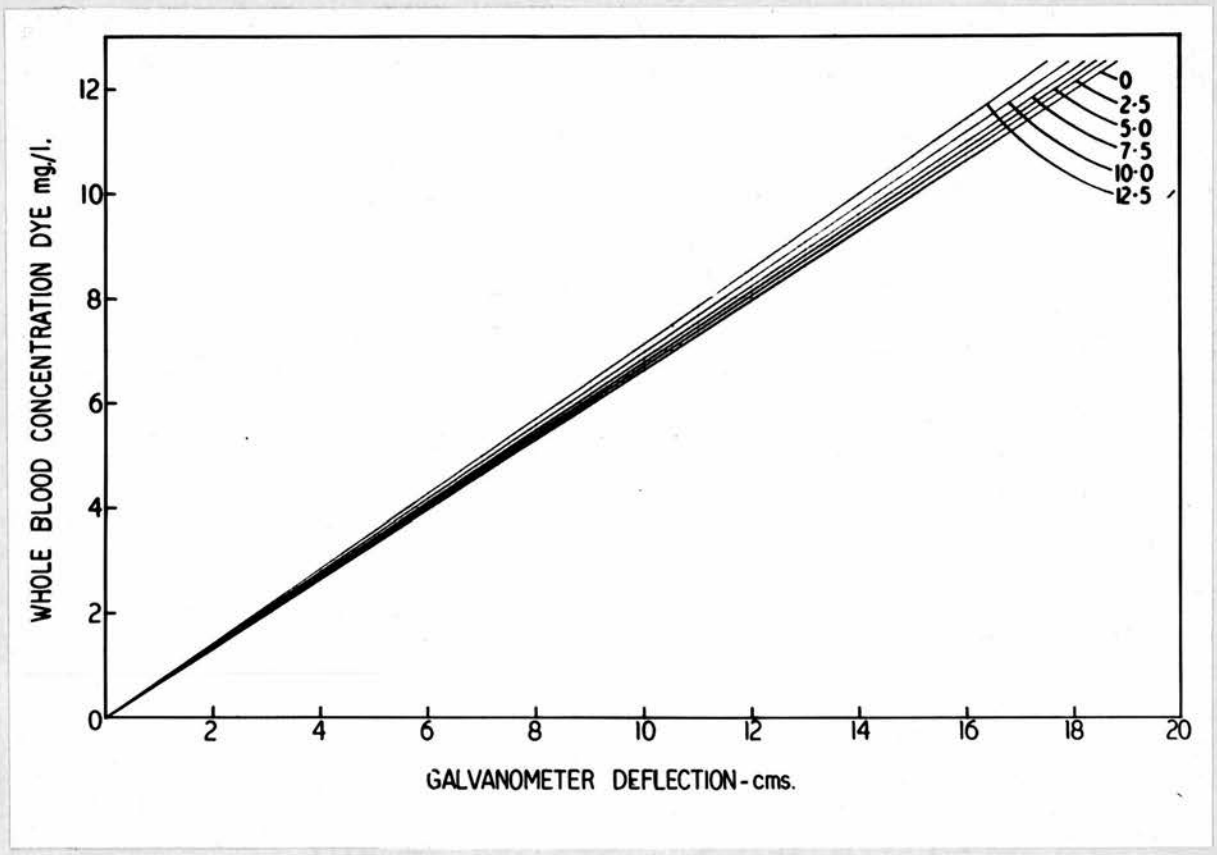

Figure 8: Effect of increasing background dye concentration on densitometer response

given over a similar time period, the equivalent of the procedure in a dye-Fick study, the ultimate concentration never exceeded two milligrams per litre. In the present study the peak concentrations of all the cardiac output dye curves averaged 6.08 mg./l. Using the data plotted in figure 8, if, at the end of an investigation, there were 2.5 mg./l. circulating background dye, the error of the deflection at a peak concentration of 6.08 mg./l. would be only one per cent., and the error of curve area would be considerably smaller.

Many previous instruments have suffered from the disadvantage that their lumens have been unduly large, thus increasing the volume-flow ratio and compromising the dynamic response characteristics. The Waters XC-250A however, has a lumen of only 0.02 ml. As mentioned earlier, the thinner the layer of dye-blood mixture presented to the photocell, the smaller are the changes in light transmission for a given amount of dye. Thicker layers, however, will increase the interference by unwanted non-specific background optical density influences. Fortunately, the cadmium-selenide photocell used in the Waters XC-250A is very sensitive, so that the cuvette volume can be kept small, the dye dosage at a minimum, and yet the photocell output does not require amplification. The resistance of a cadmium-selenide cell varies in relation to light intensity and its fixed external resistance (Nilsson, 1960). In the X-250A the system has been carefully balanced to achieve an almost linear output by connecting the photocell to an external resistance of 22,000 ohms, using a moderate light intensity.

Selenium cells in general do not have their response maxima at 800 mμ, but at a lower wavelength. Filters are therefore necessary to obtain the isosbestic point, although sensitivity is sacrificed somewhat in the infrared region. The cadmium-selenide photocell, however, produces approximately 65 per cent. of its response maximum at 800 mμ (Castillo, Zarnstorff and Gilson, 1961) giving adequate sensitivity therefore.

A final feature in favour of the XC-250A is that it is easily manipulated very close to the patient, thus reducing the length of catheter needed between the cuvette and the patient, and keeping the volume of the system as small as possible.

The Method of Withdrawal

The transmission of whole blood flowing in a cuvette varies with the rate of flow (Kramer, 1935, 1950; Pappenheimer, 1941; Opitz, 1948; Wood, 1950; Zijlstra, 1953). The nature of this phenomenon is not fully understood, but is generally explained as being due to erythrocytes being concentrated in the centre of the stream due to laminar flow through the cuvette (Wever, 1953, 1954). As whole blood does not obey Beer's law with respect to concentration changes, the reciprocal changes of concentration and optical depth brought about by this axial concentration of the haemoglobin do not cancel each other. The effect is greater as the cuvette becomes smaller and the light more parallel (Kramer, 1950). For the above reasons it is essential in any dye-dilution cuvette to keep the flow absolutely constant, especially in a monochromatic densitometer,

where connection of red and infrared cell outputs against each other to cancel this effect to some extent is not possible (Nilsson, 1960).

Various methods of passing blood through the cuvette have been employed since the first use of continuous recording techniques. These have varied from relying on the arterial pressure head itself to drive the blood through the catheter, tubing and cuvette (Falholt and Kaiser, 1955), suction provided by a falling column of mercury (Friedlich et al., 1950; Shadle et al., 1953), to withdrawal by a vacuum supply (Falholt and Kaiser, 1955; Nicholson et al., 1951). With the appreciation of the absolute necessity for maintenance of steady flow through the cuvette, and the need for high flow rates to achieve good hydraulic features, these methods have become obsolete. Many of them suffer from the severe disadvantage that flow rate falls off progressively, or is not maintained absolutely constant (Friedlich et al., 1950; Shadle et al., 1953). They also render it impossible to return the blood to the patient after inscription of the curve, thus making repeated curves impracticable due to progressive blood loss. Moreover, depending on the volume and length of the catheter system, one may wish to use varying withdrawal rates.

To achieve absolutely constant withdrawal rates, repeatable between curves, curves and calibration, and between different patients, with a selection of a wide range of flowrates, a Harvard 600 - 900 single infusion-withdrawal pump employing a 1500 r.p.m. motor (Harvard Apparatus Company Inc.) and using a 50 ml. Luer Lock syringe was chosen.

It was decided, after theoretical consideration of the optimum volume-flow ratio, to withdraw blood from the arterial system at 38.0 ml./min. Obviously this withdrawal rate is ideally suited only to the catheter-cuvette system employed in this study, as will be discussed later, although the pump could give a wide range of flowrates suited to other catheter-cuvette combinations.

Repeated estimations of withdrawal rates showed that the speed was constant and repeatable up to the maximum viscosity to be expected in the patients studied, as assessed by the haematocrits (Table 2).

TABLE 2

P.C.V. 30%

Time in Seconds	Volume in Millilitres
15.8	10
15.8	10
16.0	10
15.8	10
15.7	10
15.7	10
94.8	60

Pump withdrawal rate is 38.0 ml./min.

P.C.V. 40%

Time in Seconds	Volume in Millilitres
15.7	10
15.7	10
15.9	10
16.0	10
15.9	10
15.7	10
94.9	60

Pump withdrawal rate is 37.9 ml./min.

P.C.V. 50%

Time in Seconds	Volume in Millilitres
15.9	10
15.8	10
15.7	10
15.8	10
15.9	10
15.7	10
94.8	60

Pump withdrawal rate is 38.0 ml./min.

P.C.V. 60%

Time in Seconds	Volume in Millilitres
15.7	10
15.9	10
15.8	10
15.9	10
15.7	10
15.7	10
94.7	60

Pump withdrawal rate is 38.0 ml./min.

The Tap and Flushing Assembly

Many previous systems for dye-dilution studies have suffered from the disadvantage that the cuvette, tubing and catheter could not be adequately flushed between curves. This is essential when numerous rapidly-repeated curves are performed.

Initially, a system of taps, drips and tubing was chosen to allow ready flushing. Unfortunately, the standard spring-loaded taps available were found unsuitable (H.G.H. Instruments, London). At the high negative pressures necessary to achieve a suitable linear velocity through the cuvette, these taps invariably leaked. Air then entered the withdrawal syringe, causing the withdrawal rate to fluctuate due to the expansion of the air bubbles within the syringe. Moreover, the presence of small air bubbles in the syringe made return of the blood to the subject's arterial

system a dangerous procedure with a risk of air embolism occurring. Hansen taps, made to a specified design by Ole Dich of Copenhagen, and optically ground to achieve complete freedom from leaks, were obtained, thus overcoming this problem.

The tap assembly chosen for this study is illustrated in figure 9. In certain studies alternate measurements of pressure and flow may be required via the same arterial catheter. In such cases a combined tap assembly such as that illustrated in figure 10 can be used; but in such a system blood has to be withdrawn via an extra right angle bend in the tap assembly if a straight-through pressure channel is maintained. This reduces the maximum suction rate possible which would increase curve distortion by the sampling system. The combined system, however, overcomes the difficulty of having to remove the catheter mounting from the dye assembly, and attach it to the manometer system. For convenience it would also be desirable to have a sampling tap between the cuvette and the catheter. In the dye-Fick study however, convenience was sacrificed to achieve optimum hydraulic characteristics for the dye-dilution system, and the tap arrangement illustrated in figure 9 was used, with its separate pressure and flow assemblies, and in which, moreover, it was necessary to disconnect the catheter mounting to obtain arterial samples.

A further difficulty initially encountered with rapidly repeated curves in the same patient was a clotting problem. A thin fibrinous deposit tended to form in the cuvette and catheter lumen, thus interfering with the withdrawal rate and causing cavitation in the pump syringe.

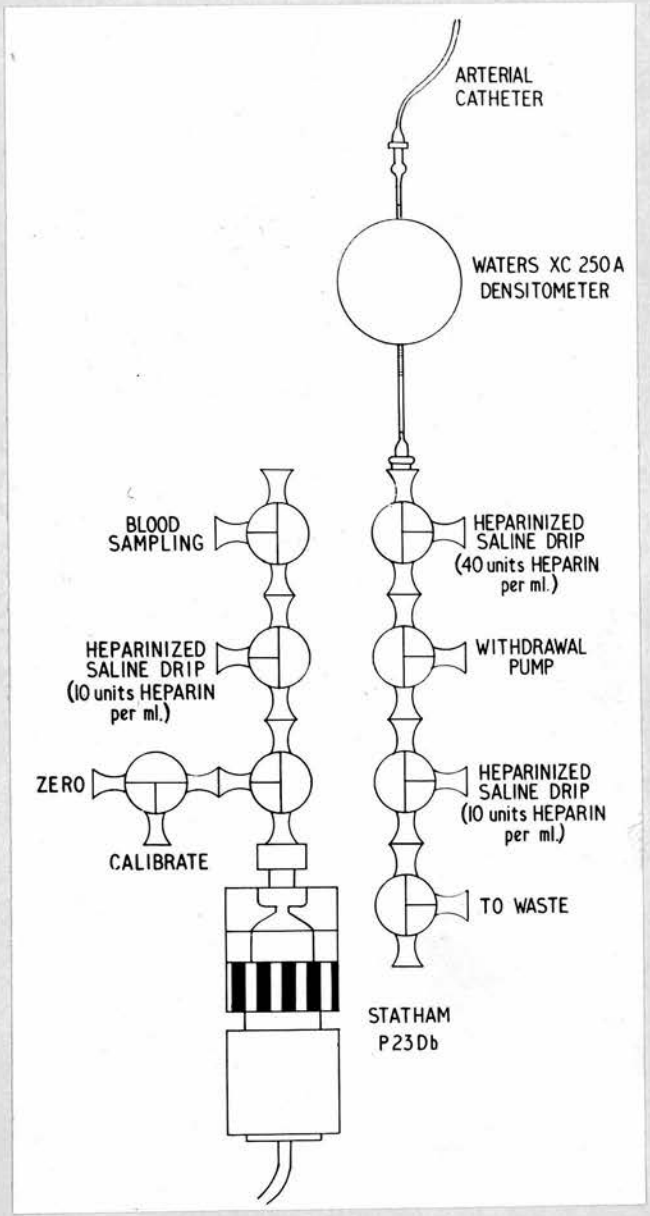

Figure 9: Separate arterial pressure and dye withdrawal tap and drip assembly

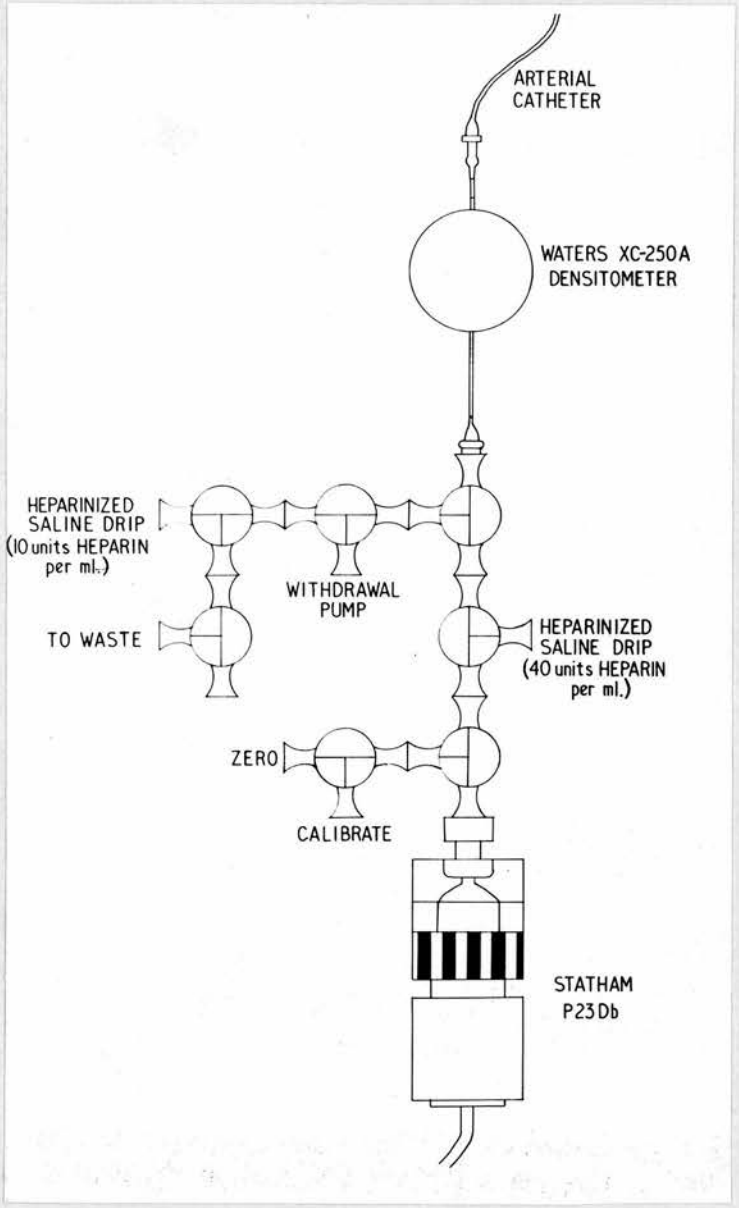

Figure 10: Combined arterial pressure and dye withdrawal tap and drip assembly

A more serious effect of this was that the fibrinous coating interfered with the light transmission of the cuvette, causing progressive damping of the curves and erroneous curve areas. This was overcome by using 40 units of Heparin per millilitre of normal saline in the drip nearest to the cuvette (figure 9). Only 10 units per millilitre were used in the other drip which functioned as a reservoir for flushing the syringe. It is obviously undesirable to load a patient with Heparin during any procedure, but thrombin times done by the toluidine-blue thrombin clotting time method (Holmgren and Wilander, 1937; Biggs and Macfarlane, 1957), were performed at the beginning and end of the investigation, and usually showed only insignificant prolongation. Where the thrombin time was slightly prolonged, an appropriate dose of Hexadimethrine bromide (Polybrene) was administered, and the removal of the catheters delayed to allow the temporary coagulation defect time for correction. No sequelae were encountered from this occasional temporary disturbance of the clotting mechanism.

The Dye Injection Assembly

One of the most important measurements in the accurate calculation of cardiac output by the dye-dilution method is the volume of the injectate. A slight error in this introduces large errors in the calculated output. Initially, systems for dye injection were somewhat inferior, for the large volumes of injectate delivered probably compensated for volumetric errors which would have been quite considerable in an injectate of small volume, as employed in this study. The commonest system used was a three-way

stopcock in which, after injection of the dye, the tap was turned on the stopcock, and the dye remaining in the catheter immediately flushed through with saline. At high sensitivities this saline-flush method could introduce considerable errors into monochromatic densitometric studies, as saline changes the optical density of blood, and acts as an indicator itself (Sinclair, Sutterer, Wolford, Armelin and Wood, 1960; Sinclair et al., 1960, 1961). Using indocyanine green, and the Waters X-250A densitometer, it is essential to inject by displacement, and avoid the saline-flush method.

The injection system employed in this study is a Horwell pipetting unit with a two millilitre syringe, which had the added advantage of reloading itself almost instantaneously from a dye reservoir flask after delivery of the injection. The syringe was mounted in a cradle incorporating a spring-loaded manually-operated lever which depressed the plunger. The volume of the injectate has been found to be reproducible with extreme accuracy, in the present study averaging 1.450 ml. (range 0.955 to 1.569 ml.), depending on the syringe used. Repeated injections from the same syringe agree to within 0.01 ml. over four consecutive injections (S.E. of mean - 0.051 per cent.; S.D. - 0.176 per cent.). In the standard model of the Waters X-250A dye-dilution assembly, the control unit has a manually controlled button which marks the injection time on the recorder trace. This system introduces considerable errors in recording the timing and duration of the injection. Hence, a microswitch was fitted to the syringe injector in parallel with the marker button, so that the duration and timing

of the injection was accurately inscribed on the trace.

Ideally, injection should be instantaneous; in practice, however, a finite time is necessary, and a compromise must be reached. If the mean transit time through the system is long in comparison with the mean time of the injection, no error will ensue. If, however, the mean time of the distribution of the injection is significantly long in comparison with the mean transit time through the system, the calculation of the mean transit time, and hence central blood volume, will be erroneous (Zierler, 1962 b). From the results of model studies, Hoffman and Shillingford (1957) claim that the estimation of cardiac output is affected by an injection time greater than half the appearance time. Theoretically it is obvious that the mean transit time will be affected by a long injection, thus invalidating central blood volume values, while cardiac output measurement could be affected by a very long injection due to the interference of recirculation with the recognition of the primary curve on the semilogarithmic replot. Marshall, Birkhead and Wood (1958) performed studies in dogs demonstrating the effects of varying injection duration from 0.3 - 6.0 seconds or more. The calculated cardiac output was unaffected, but the appearance time, buildup time, mean transit time and central blood volume were all distorted by long injections. With all the precautions taken in the present system to obtain undistorted curves, it was therefore essential to achieve rapid injection, and the duration in the dye-Fick comparison varied between 0.2 and 0.6 seconds. There are, however, further improvements which could have been made in the injection mechanism, which will be discussed in a later section.

The injection dye dosage is obviously a function of the sensitivity of the detecting instrument, and it is desirable to keep dosage to a minimum. Using indocyanine green, Evans blue and indigocarmine, Birkhead, Fox and Wood (1957) have shown that doubling and quadrupling the dose of dye does not affect the accuracy of the estimation of cardiac output, which is not surprising. To economise, and to reduce the level of background dye as far as possible however, individual injection dosage has been kept to a minimum in this study, and with central venous injection and aortic sampling, the dye dosage used averaged 2.624 mg.

The volume of the injection has also been kept small. The flow being measured is increased to the extent of the volume injected, which is an important objection to indicators requiring large injection volumes, quite apart from the longer time necessary for their injection. Errors of the method will therefore be minimised if the injection volume is small, compared with that of the vascular bed between the sites of injection and sampling (Gray and Paton, 1949).

The final problem to be resolved with reference to dye injection is its site. Formerly, dye injections were routinely given into peripheral veins, but, following the introduction of pulmonary artery injection (Ebert et al., 1949), a series of studies appeared, some of which were technically inadequate (Coe, Best and Lawson, 1950; Lawson, Shadle, Coleman and Holtgrave, 1954), setting out to show discrepancies in cardiac output calculated from curves with central and peripheral injections. Some suggest an actual discrepancy (Heller et al., 1953; Gunnells and

Gorten, 1961). The Mayo group, however, have shown that there is in fact no error (Hetzel et al., 1954; Carter, Swan and Wood, 1959; Bassingthwaighte et al., 1962).

In theory the concept is not a difficult one. Rossi et al. (1953) have shown that the dilution principle is valid for the measurement of cardiac output, no matter how slowly the indicator is introduced, because turbulence occurs in the ventricles. Speed of injection is relevant to injection site because laminar flow in the veins causes attenuation of the dye bolus en route to the heart. Whereas injection into a vein may take only a fraction of a second, its arrival at the right atrium may be spread over several seconds. Were there not the problem of excluding recirculation, this would not introduce any error in the calculation of cardiac output (Rossi et al., 1953), but since the calculation of curve area depends on the successful separation of recirculating dye by extrapolation of an exponential curve, the effect of laminar flow at the point of injection may be to distort the curve so badly that extrapolation becomes impossible. Mixing in the circulatory system cannot be regarded as a uniform process. The characterisation of the indicator-dilution curve by two near-Gaussian components (Korner, 1961) has provided a qualitative picture of the dispersion process, which is in good agreement with the experimental facts. This concept explains the change in symmetry of the curves with changing distance between injection and sampling sites. When a very small distance between the two sites is used, this has the advantage of minimising the likelihood of any hidden recirculation.

Moreover, Gunnels and Gorten (1961) have suggested that part of the indicator may be held back at the site of injection into a peripheral vein by venospasm or retrograde flow, which would further disperse the dye on its first circulation. For these reasons injections in this study were made centrally, and high aortic sampling was also used, as will be described later.

The Recorder

Certain systems used in the recording of dye-dilution curves suffer from the disadvantage of using direct writing recorders, many of which have considerable inertia, causing damping of the curves. Galvanometers with poor response times cause distortion effects, although it is undoubtedly true that the distortion introduced by hydraulic effects usually far outweighs any caused by inadequate response of the recording system (Fox, Sutterer and Wood, 1957). Many recorders also have fixed paper speeds, which means that during exercise, or in patients with valvular incompetence, curves with very short or long time-bases respectively would not be as accurately measurable as they are with a recorder in which the paper speed can be adjusted.

In the present study a New Electronics Products ultraviolet recorder (Type 1185) was used with N.E.P. mirror galvanometers (Type BB 30). Their frequency response with optimum damping resistance (64.5 per cent. of critical) is flat within two per cent. up to 19 c/s, and they are linear within two per cent. up to ten centimetres deflection on either side of zero. Although this frequency response may seem unnecessarily

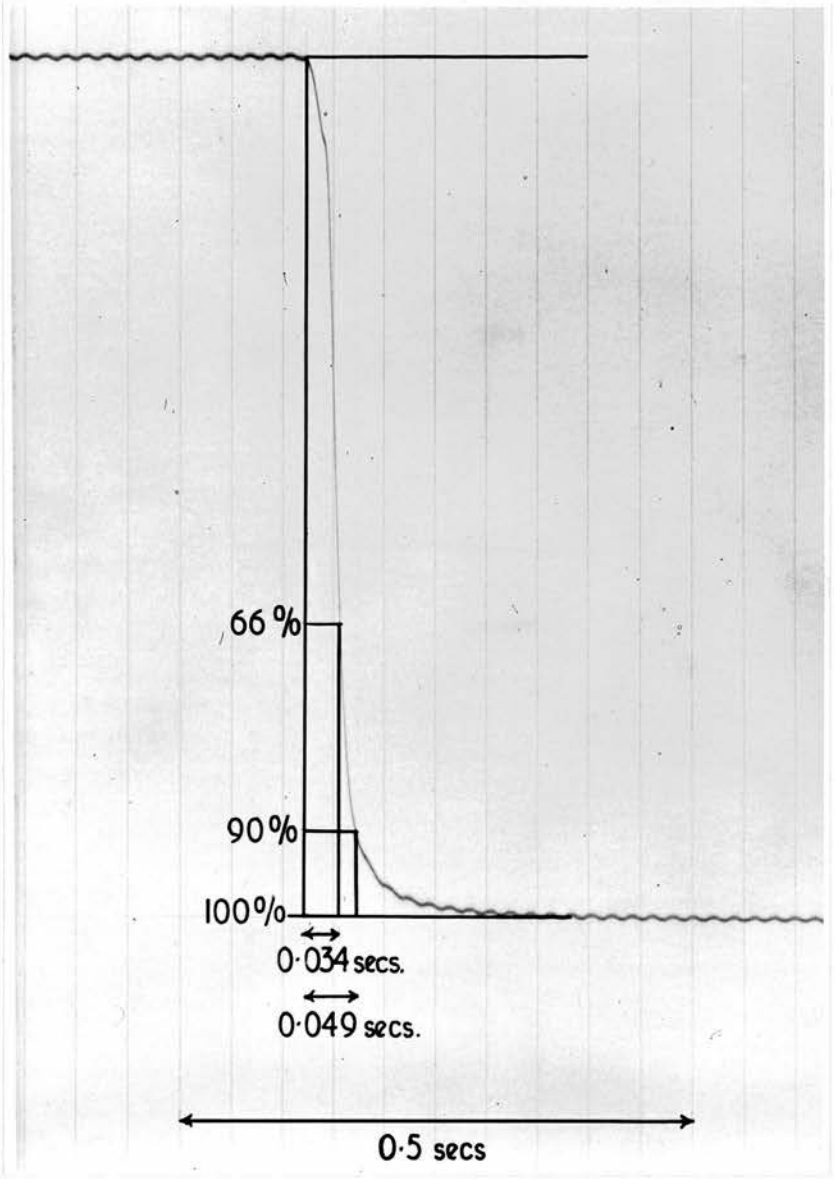

Figure 11: Electrical response curve of detecting-recording system

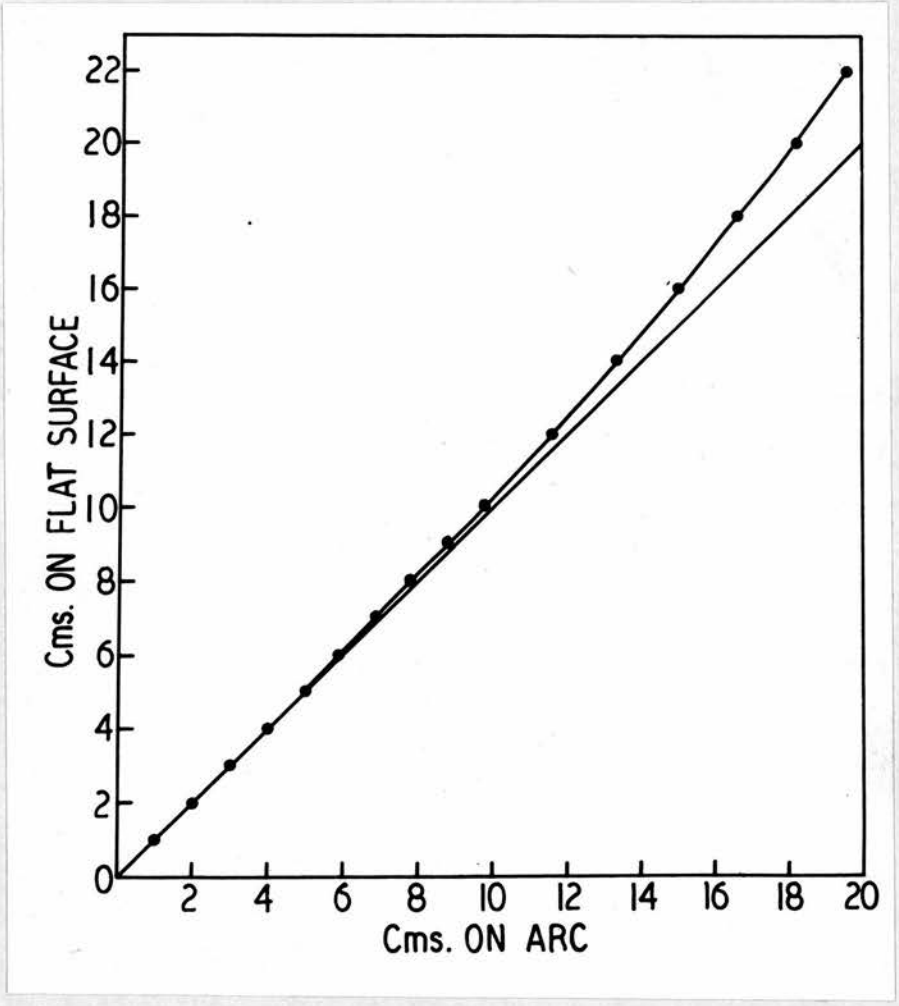

Figure 12: Distortion caused by recording on flat surface with optical beam of 35 cm.

good for dye-dilution recording, they were in fact chosen for their great sensitivity. Their D.C. sensitivity, using the optimum value of damping resistance on an optical arm length of 35 cm. is: Current sensitivity - 1.0 μA/cm.; Voltage sensitivity - 40 μV/cm. The paper width is 30 cm. and there are 21 different paper speeds, between 0.084 and 203.2 mm./sec., a range which more than embraces any paper speeds needed in dye-dilution curve recordings. An automatic timer, operating a rotating mirror in the path of an ultraviolet beam, inscribes vertical lines on the trace every half-second, thus facilitating measurement of the dye curves at accurate time intervals. The time markers operate every 1.0 or 2.5 seconds at the slower paper speeds, and every 0.1 or 0.25 seconds at faster paper speeds.

The overall electrical response time of the photocell, control unit and galvanometers was found to be 0.034 seconds for 66 per cent. response and 0.049 seconds for 90 per cent. response (figure 11).

A factor which had to be taken into consideration in the overall system was the alinearity produced by the galvanometer beam writing on a flat instead of a curved surface. With an optical arm length of 35 cm., this was calculated trigonometrically, and is illustrated in figure 12. This alinearity could introduce considerable errors into the accurate measurement of dye curves; how it was compensated for will be described in the following section.

The Linearity of the System

It requires no explanation that the relation between dye concentration

in blood and the deflection recorded on the ultraviolet trace should be an absolutely linear one for the accurate quantitative recording of cardiac output from dye-dilution curves. In the system used, three potential sources of alinearity are present:

i) the alinearity of the photocell resistance in response to decreasing light intensity

ii) the distortion by the galvanometer

iii) the distortion of inscribing curves on a flat surface instead of on a curved surface with a radius of the optical arc length (35 cm.).

As pointed out earlier, the linearity of the relation between light intensity and the resistance of a cadmium-selenide cell is largely dependent on the value of the resistance in series with the cell. In general, a linear photocell response is best obtained with a medium external resistance and moderate light intensity. The limiting values, however, vary rapidly between different types of cells, and to a lesser extent between individual cells of the same type. The manufacturers of the Waters X-250A densitometer have so arranged the light intensity and external resistance to give an almost linear cell output, as discussed under the description of the densitometer. Figure 13, obtained from the manufacturers, shows the almost linear relation between resistance and light density, obtained with the photocell (CL404) over the range 0 - 24 mg./l. dye concentration in blood, with a constant 20 volts applied at an ambient temperature of 25°C.

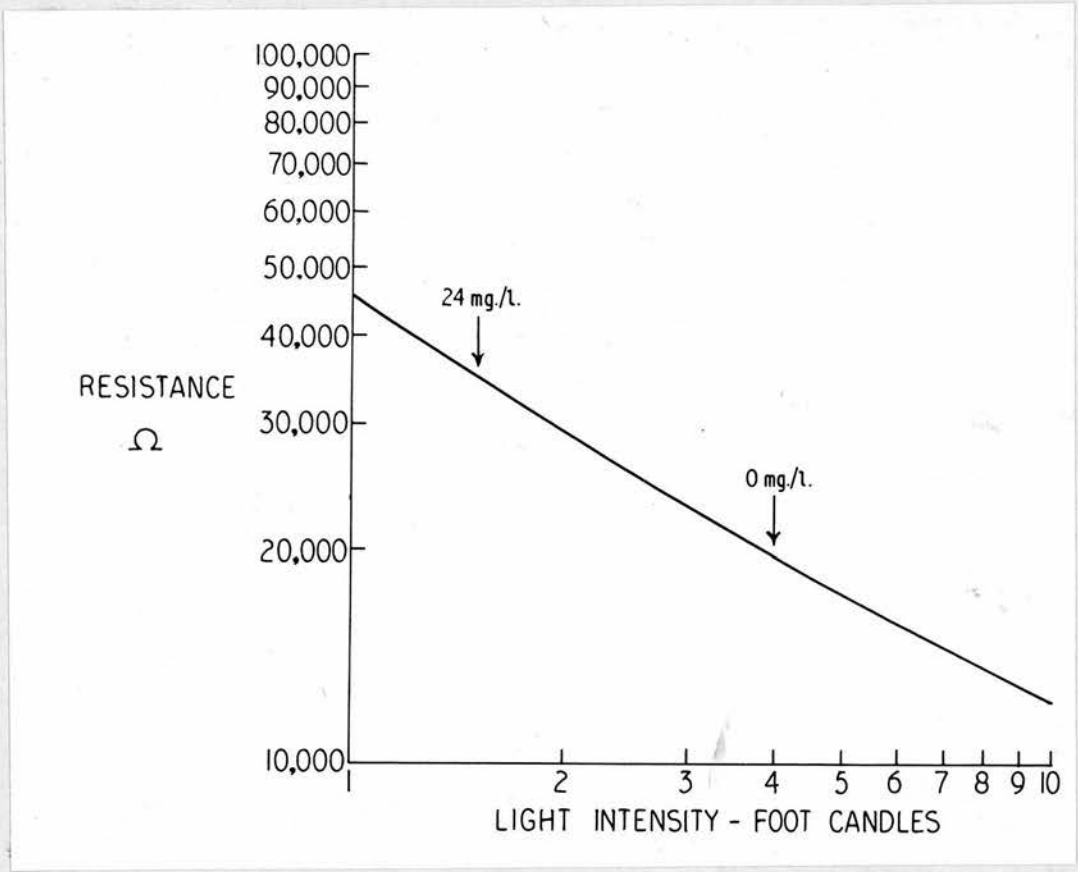

Figure 13: Effect of decreasing light intensity on photocell resistance

The N.E.P. mirror galvanometers used in the recorder are linear within two per cent. up to ten centimetres deflection either side of zero with an optical arm length of 35 cm. Since the average dye curve is not more than 15 cm. from baseline to peak concentration, this also produces only slight distortion.

The distortion effect of the light beam writing on a flat surface will produce quite marked alinearity (figure 12). This, however, was counter-balanced by the distortion caused by the slight alinearity of the photocell resistance and galvanometer torque in the opposite direction, by adjusting the zero position of the light spot to one side of the centre of its arc.

A Vibron electrometer was connected in parallel with the mirror galvanometer of the ultraviolet recorder. A controlled voltage was applied to both instruments simultaneously, and reading taken from both. The range of galvanometer deflections more than embraced the range used in recording an ordinary dye curve, and the baseline used was eight centimetres from the lower edge of the paper, as in the recording of dye curves. It is evident in figure 14 that the distortion produced by the recording system, which is the resultant of the galvanometer torque and arc distortion, is less than 1.5 per cent. at maximum deflection. Arc distortion therefore slightly overcorrects the distortion caused by the galvanometer alinearity, but this would have an insignificant effect on curve inscription.

The resultant of the photocell, galvanometer and arc distortion

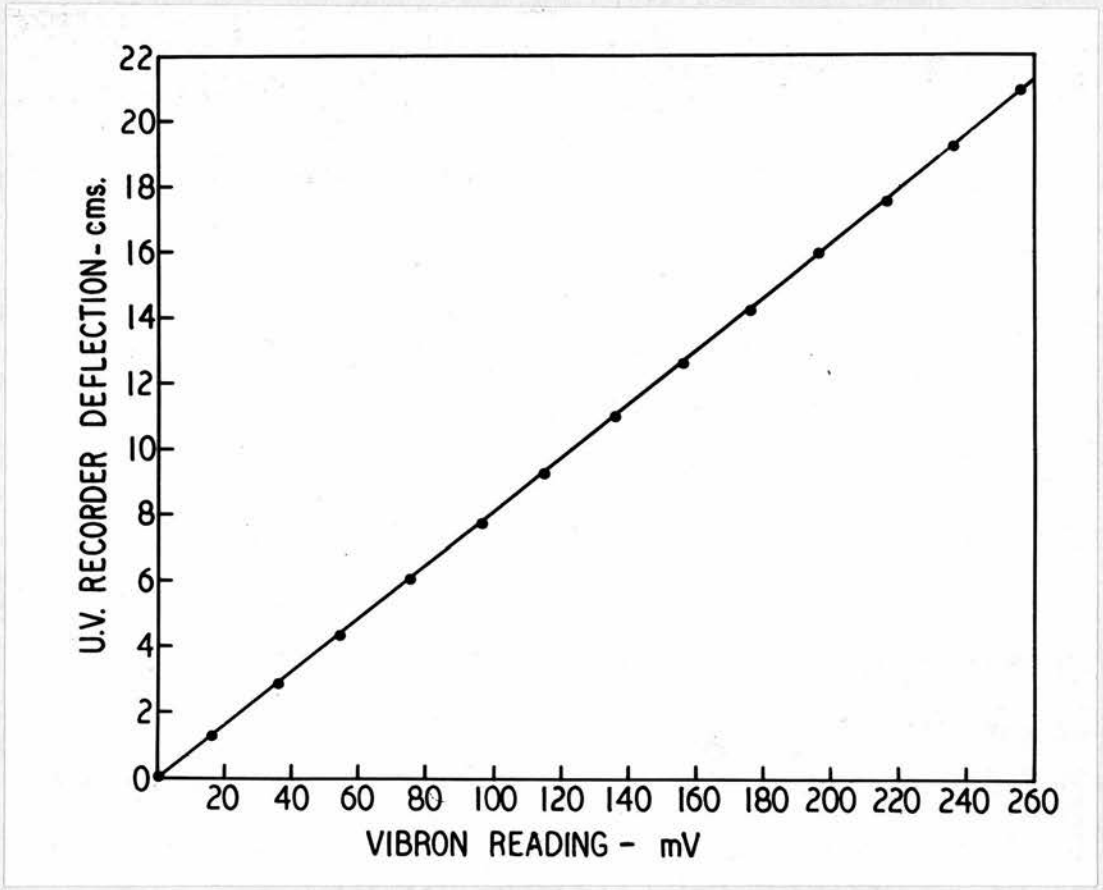

Figure 14: Ultraviolet recorder deflection to fixed millivolt input

sources of alinearity were assessed by a calibration curve extending from 0 - 60 mg./l. of dye in blood at a low sensitivity adjustment (sensitivity one), so that the entire range of deflections would fit on the recorder paper. Allowances were made for the large amounts of dye which were added to the high concentration blood samples, by making up various concentrations of dye. The volumes added to each sample therefore were all small and approximately the same (Table 3).

TABLE 3

Concentration of dye in blood in mg./l.	Volume of dye added in ml.	Concentration of dye used in mg./ml.	U.V. deflection in cm. at sensitivity 1
0	0		0
5	0.028		1.68
10	0.056	4.5	3.32
15	0.083		4.94
20	0.055		6.62
25	0.070		8.25
30	0.083		9.79
32.5	0.090	9.0	10.62
35	0.097		11.31
37.5	0.104		11.94
40	0.111		12.79
42.5	0.059		13.95
45	0.063		14.52
47.5	0.060	18.0	15.30
50	0.069		16.01
55	0.076		17.19
60	0.083		18.45

It can be seen that the overall linearity of the system is quite adequate up to a concentration of approximately 25 mg./l. (figure 15). Dye curves used in cardiac output estimations have peak concentrations

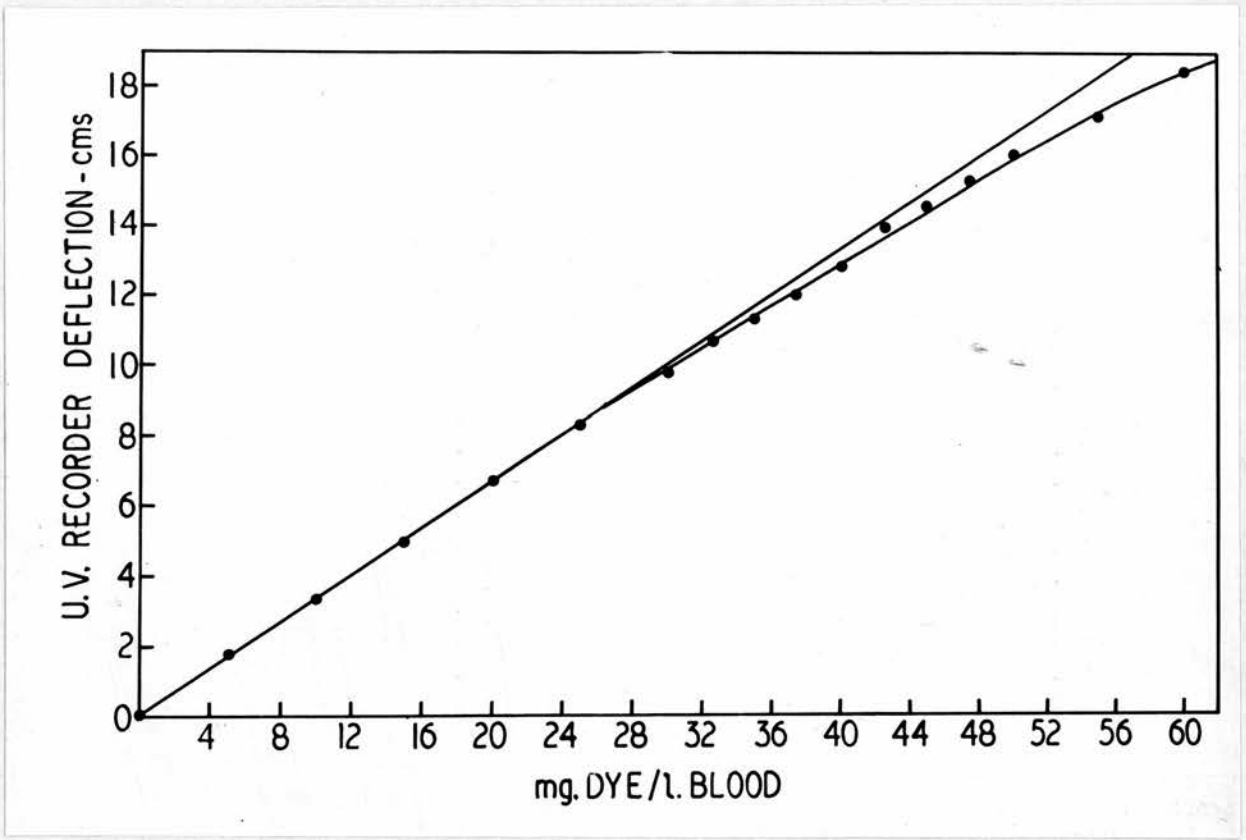

Figure 15: Calibration curve of dye in blood from 0-60 mg./l. at sensitivity one

averaging 6.08 mg./l., which is well within the linear range therefore.

The question of alinearity due to rising background dye levels has been discussed in a previous section, where it was explained how the adjustable light intensity overcame this problem.

The Sampling System and the Problems of Dynamic Response Characteristics

Most earlier dye-dilution studies employed an arterial needle for sampling from the brachial, radial or femoral artery. In this study, however, a fine nylon catheter was introduced by the Seldinger technique, and passed so that its tip lay in the aortic root. Preliminary studies with radio-opaque catheters inserted by the same technique showed that the catheter tip always attained the desired position. Radio-opaque catheters of dimensions suitable to achieve the required volume-flow characteristics in the present system were not commercially available, and it was therefore decided to change to non-opaque catheters. On the basis of this earlier experience with radiographic screening to check the position of the catheter tip, and by always checking the wave form characteristics before starting a study, these 55 cm. nylon catheters can be said with reasonable confidence to have attained the same position in the aorta.

The reasons for this central sampling are the following. As originally shown by Kinsman et al. (1929) in their model studies, and by Beard et al. (1951) in studies on patients, the mixture of dye and blood is homogeneous on leaving the left ventricle after injection on the right side of the heart, and sampling from any artery, large or small, proximal or distal, should give the same values for cardiac output. The

concentration of indicator, as it is ejected from the heart, however, varies with each heartbeat. During the traversal of the arterial system, these changes are damped out by the smearing effect of the arterial tree (Rossi et al., 1953; Schambye, 1953; Taylor, 1954; Sheppard, 1954, 1957; Grace et al., 1957; Fox et al., 1957; Milnor and Jose, 1960). Similarly, the concentration-time relationships of the curve are modified by traversal through the catheter, in which smearing also occurs, due to axial flow being faster than peripheral. This is mainly an appearance delay, but continuous recording systems record mean concentration, unlike intermittent sampling methods which measure mean dye flux, and this causes some dispersion of the curve with depression of the peak concentration as well. Sampling at peripheral sites, however, introduces a smearing effect which cannot be controlled, and this is the main justification for sampling through a centrally-placed catheter, where there is at least a prospect of understanding what the catheter smearing effect may be (Sheppard, Jones and Couch, 1959; Sherman, Schlant, Kraus and Moore, 1959).

Burger et al. (1956), and van der Feer (1958) have shown mathematically that for an absolutely accurate measure of mean transit time there must be turbulence at both the injection and sampling sites, resulting in a homogeneous velocity distribution. Since turbulence is present only in the initial part of the arterial system, and not in peripheral arteries such as the brachial or femoral, the parameter of mean transit time can be accurately assessed only with high aortic sampling.

The indicator-dilution technique requires, as one of its criteria, a constant flowrate or a constant indicator concentration in the system in which flow is being measured (Hamilton et al., 1932; Meier and Zierler, 1954; Zierler, 1958). Since indicator concentration is changing rapidly at the sampling site, a systematic error may be expected to result, when sampling at a constant flowrate over the sampling period (time averaged sampling), rather than at a rate proportional to the total flow past the site at all phases of the sampling period (volume averaged sampling) (Marshall, Allwood, Keck and Shepherd, 1961; Bassingthwaighte et al., 1962). Flow in the arterial system is pulsatile, and the condition of constant flow is never fulfilled. The variations in flow, however, are not very great in peripheral arteries, although their amplitude is considerable at central sites such as the proximal aorta (McDonald, 1960). It seems reasonable, therefore, that random and systematic errors may occur due to violation of the indicator-dilution principle by central sampling (Marshall et al., 1961).

Many workers have found that the areas of centrally sampled curves are roughly equal to the areas of those sampled peripherally, but the variations were large (Theilen, Gregg, Paul and Gilford, 1955; Keys, Swan, Hetzel and Wood, 1956; Fritts et al., 1957; David, Swan and Wood, 1958; Emanuel, Lacy and Newman, 1959; Lange, Smith and Hecht, 1960; Sleeper, Thompson, McIntosh and Elston, 1962). Recently, Bassingthwaighte et al. (1962), in a more careful study on dogs, using indocyanine green, have shown a slight but insignificant difference

between cardiac outputs calculated from simultaneous centrally and peripherally sampled dye curves.

Apart from the above considerations, as explained with reference to the desirability of central injection, the advantage of a central sampling site is also that it minimises early contamination of the curve by recirculating indicator. This causes the recirculation to occur lower on the downslope of the curve, and so facilitates more accurate extrapolation (Falholt and Fabricius, 1956; Marshall et al., 1961).

The contour of a dye curve is determined by the volume-rate of flow through the vascular system, the volume of the vascular bed between injection and sampling sites, the distribution of the long and the short circulatory pathways through the system, and other less well understood factors (Rossi et al., 1953; Sheppard, 1954). The instrumentation should be designed to reproduce as faithfully as possible the contour of this curve without adding yet another factor to those already mentioned, which will influence its true shape. Unfortunately, sampling, detecting and recording systems inevitably introduce some degree of distortion, and the best that can be achieved is to minimise this distortion as far as is practicable. The theoretical ideal is not always possible, as catheters have to be of a certain minimum length to sample from the desired site; they must be of a suitable bore to enable satisfactory suction rates, and these rates must not be excessive, or blood loss will influence the very parameter being measured. While distortion of the curve by the sampling system departs from the concept of representative sampling, it changes only the shape and time relationships, and not the total area

under the curve (Marshall et al., 1961). Since area is the only parameter of the curve needed for the calculation of cardiac output, it may seem unnecessary to strive for ideal dynamic response characteristics. However, because cardiac output is only one of the many parameters of value obtained from a dye curve, it is desirable to obtain an accurate assessment of the others, all of which are very much affected by these considerations. They include central blood volume, slope, peak concentration, appearance time, buildup time, mean transit time, the assessment of valvular disease, heart chamber volumes, and the recognition of cardiac shunts.

It is necessary to assess the dynamic response characteristics and distortion produced by the system used, albeit slight. Laminar flow, in which velocity bears a parabolic relation to distance from the axial stream, would have predictable effects on indicator dilution curves (Rossi et al., 1953; Taylor, 1954; Sherman et al., 1959). Such theoretical predictions probably do not apply to blood flow in tubes however, because the velocity profile of plasma is not parabolic, and differs from that of erythrocytes (Coulter and Pappenheimer, 1949; Rossi et al., 1953). An empirical approach to the problem is therefore necessary (Fox et al., 1957; Milnor and Jose, 1960).

In most sampling systems laminar flow exists. Factors which will flatter the velocity profile produced by this laminar flow, and thus reduce the degree of longitudinal dispersion, will improve the dynamic response. Where longitudinal dispersion is present, factors which will

reduce the time required for the fluid column in which this dispersion exists to pass the detecting photocell, will also improve the dynamic response of the system. The physical dimensions of the connecting tubes and cuvette lumen, and the rate of flow through them, affect the dynamic response by altering these factors. Turbulence over the full width of the stream, as is produced in the Colson densitometer by incorporation of an S-bend in the pathway proximal to the detection chamber, would produce an essentially flat velocity profile, and hence should theoretically improve the system. However, the mixing action of turbulent flow acts in the longitudinal as well as in the radial direction, so that this type of flow also has a longitudinal dispersing action (Taylor, 1954).

Poor dynamic response is determined chiefly by the hydraulic components of the system, namely the volume of the detecting chamber, the length and internal diameter of the catheter, and the linear velocity of blood flow through the catheter, rather than by the dynamic response characteristics of the electrical components of the transducer and recording system (Fox et al., 1957; Wood et al., 1957). Numerous workers have shown that the distortion effects can be minimised by obtaining the highest linear velocity possible through the sampling and detecting system, taking into account the limitations imposed by catheter length, bore and withdrawal rate (Rossi et al., 1953; Fox, Sutterer and Wood, 1955; Fox et al., 1957; Lacy, Emanuel and Newman, 1957; Dow, 1958; Sherman, Schlant, Kraus, Moore, Haynes and Dexter, 1958; Sherman et al.,

1959; Sheppard et al., 1959; Edwards, Cheesman and Wood, 1959; Milnor and Jose, 1960; Marshall et al., 1961). The ways in which this increased linear velocity improves dynamic response are as follows:

i) Delays in the various landmarks of the curve are reduced if the dyed blood at the tip of the catheter is conveyed to the detecting photocell in the shortest time possible.

ii) A high linear velocity decreases the time available for mixing during transit of the blood from catheter tip to photocell.

iii) Greater spatial separation of peaks and valleys of concentration differences along the catheter is obtained, and concentration gradients per unit length of tubing are thus reduced, serving to decrease the effect of longitudinal dispersion of dyed and undyed blood during traversal of the catheter.

iv) Rapid flow causes a greater volume of blood to flow through the detecting chamber, washing it out more completely of the sample which preceded it, and so the dyed blood actually in the chamber more accurately approaches the true concentration of dye in blood.

In the system used it was decided that, with a brachial artery puncture, the shortest catheter which could achieve aortic root sampling

was 55 cm. in length. Aiming at as low a volume-flow ratio as possible, its internal diameter and the rate of withdrawal were chosen from the suitable catheter dimensions and pumps available. The 55 cm. catheter chosen had an internal diameter of 1.0 mm., and the withdrawal rate chosen was 38.0 ml./min. The distance from the cuvette centre to the catheter tip, including the catheter, connector and adaptor, was 62 cm., and the total volume was 0.48 ml. This gave a linear velocity of 81 cm./sec. down the catheter, and a volume flow ratio of 0.76. The volume flow ratio is the volume in millilitres from the tip of the catheter to the midpoint of the optical path in the cuvette, divided by the rate of flow through the catheter in millilitres per second.

Having determined the volume flow ratio and linear velocity of the system described, it is necessary to determine its response characteristics. Fox et al. (1957) have described a method of doing this using a square wave front of dyed blood introduced at the catheter tip, with an assessment of its distortion by the system employed.

Theoretically, the recording of a perfect square wave requires, in addition to a point detector, a truly square front impulse. These criteria are most closely approached in electrical systems, and are much more difficult to approach in hydraulic systems. Even in fully developed turbulent flow of very high Reynold's number, the presence of a "laminar sublayer" at the fluid-wall interphase precludes attainment of a completely square front. The parabolic velocity profile of laminar flow introduces a time interval between the instant at which the first particle of dye

reaches the detection chamber, and the time at which only dye-laden blood overlies it. The presence therefore of laminar flow, and possibly a "laminar sublayer" are probably responsible for the "asymptotic-like" approach to 100 per cent response of the deflection of the apparatus caused by such an artificial square wave of dyed blood.

Undoubtedly, one of the chief underlying factors producing this effect, which thus worsens the dynamic response characteristics, is the longitudinal dispersion of the dye-blood mixture, caused by differences in velocity of different portions of the stream associated with laminar flow. Taylor (1954), in his experiments with water-filled rigid tubes, derived equations to predict the degree of dispersion. But, as he recognised, and as was also demonstrated by Rossi et al. (1953), these formulations cannot be applied to blood, which has complex hydro-dynamic properties.

Fox et al (1957) constructed an apparatus to deliver alternating square waves of dyed and undyed blood at variable frequencies to their sampling-detecting-recording system. Since it is difficult to determine from traces the exact instant at which 100 per cent. deflection is reached, although the amplitude of the latter is quite well-defined, the time at which a response of 90 per cent. of full deflection is attained was measured. They also calculated their instrument response to these square wave variations in dye content at increasing frequencies as amplitude response, by expressing the recorded peak-to-peak deflections as a percentage response of the instrument to a square wave of dyed

blood which was maintained for a sufficient time for the instrument to reach 100 per cent. deflection. Obviously the degree of attenuation increased as the frequency of the square waves of dyed blood increased, because the amplitude response is a function of the time that a stimulus is permitted to act.

In the system described in this study, the 66 per cent. and 90 per cent. response times were determined by a method similar to that described, and were found to be 0.309 and 2.127 seconds respectively (figure 16). Subtracting the equivalent known electrical response times of 0.034 and 0.049 seconds (figure 11), this gives a 66 and 90 per cent. hydraulic response time of 0.775 and 2.078 seconds respectively. Fox et al. (1957) derived an equation expressing the relation between the 90 per cent. response time (T_{90}), and the frequency at which the sensitivity is reduced to 80 per cent. of static sensitivity (F_{80}). The graph of these resembles a rectangular hyperbola, and the equation is:

$$T_{90} \times F_{80} = 29$$

Using this formula, and the data of a single square wave response of the system (figure 16), the frequency at which sensitivity is 80 per cent. of static sensitivity in the present system is 13.6 cycles per second.

As pointed out by Fox's group, the use of square wave input signals of increasing frequency, and the expression of dynamic response as the decrease in maximal amplitude of response, flatters the degree of actual distortion in the recorded curves. Milnor and Jose (1960)

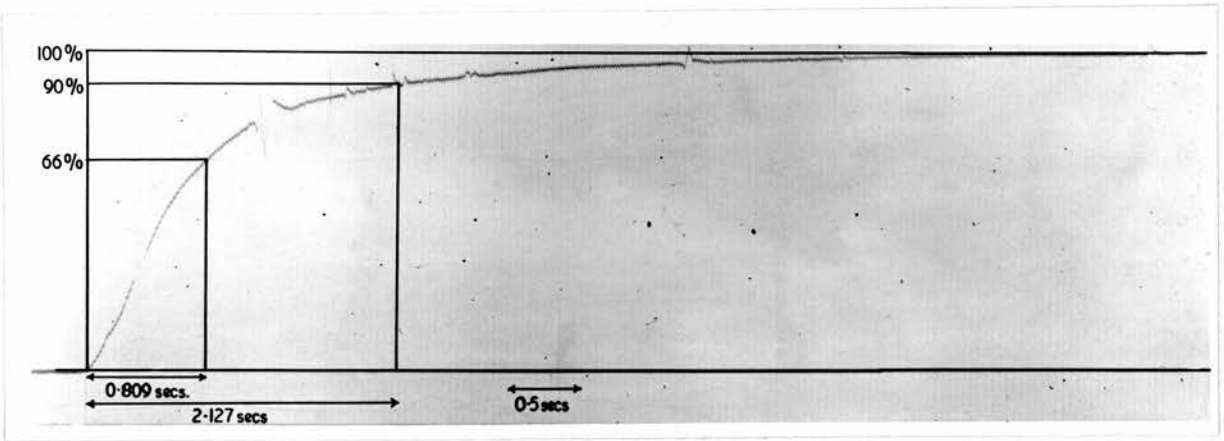

Figure 16: Hydraulic plus electrical response curve to square wave of dyed blood in sampling-detecting-recording system

criticise the method as being more appropriate for characterising electrical systems, and for giving only a qualitative indication of the distortion of true indicator dilution curves, which are very different from square waves. They therefore devised a system whereby they could measure the "true" indicator-dilution curve, undistorted as it left a mixing chamber, as well as after traversal of several sampling systems with different volume flow characteristics. They were therefore able to assess distortion effects on many curve parameters, and relate dynamic response to more than just amplitude response, as in square wave input signals. They obtained the "true" curve by directing a collimated light beam through the base of the outflow neck of the mixing flask, in which the dye curve was being fashioned, and through a filter to their phototube. They sampled their curve simultaneously within a few millimetres of this point through their several sampling systems. A "true" and a distorted curve were thus obtained, and from each they could ascertain appearance time, buildup time, mean transit time, peak concentration and slope.

Their results indicated that the volume flow ratio of the sampling system determined absolutely the hydraulic distortion effects. They ascertained from their findings the relationship between the distortion of the curve parameters and the volume flow ratio of the system, and derived regression equations for each parameter. From these equations the distortion in the present apparatus, which has a volume flow ratio

of 0.76, was determined:

$\triangle AT = 0.410 + 0.553\ (V/F) - 0.00376\ (V/F)^2 = 0.83$ seconds

$\triangle BT = 0.325 + 0.359\ (V/F) - 0.00871\ (V/F)^2 = 0.59$ seconds

$\triangle MCT = 0.140 + 0.901\ (V/F) - 0.00933\ (V/F)^2 = 0.82$ seconds

$$\frac{\text{Apparent Cp} \times 100}{\text{True Cp}} = 100\ (1.088 + 0.0203\ (V/F) - \sqrt{V/F}\ ) = 89 \text{ per cent.}$$

They concluded that a volume flow ratio of 0.5 or less was a satisfactory compromise between the theoretical optimum and the limits imposed by the experimental situation. This figure is however permissibly larger in the present system as their limit was set for peripheral sampling. Since central sampling overcomes a considerable smearing effect of the arterial tree, it compensates for the sacrifice of a better volume flow ratio obtainable by sampling through a shorter catheter, possible at a more peripheral site.

A further figure of merit for the catheter-sampling system has been suggested by Sherman et al. (1958, 1959). They submit that where V represents the volume from the catheter tip to the midpoint of the optical path in the cuvette in millilitres, and Q represents the flow through the catheter in millilitres per second, $\frac{V}{2Q}$ should be less than half the interpulse time, or the expected time of change of indicator concentration at the catheter input (i.e. once per cardiac cycle). They also advocate that the catheter tip should be as near the output of the system under study as possible, in this case the aortic root. In the present system

$\frac{V}{2Q}$ = 0.38, which represents half interpulse time at a heartrate of 79 per minute.

Two sources of distortion cannot be overcome by present systems. Where the mean concentration of dye in blood in the cuvette is recorded, as in present densitometric techniques, the mean is a spatial average taken over the entire cross section of the lumen. The indicator substance in any lamina, therefore, will tend to contribute equally to the record of mean concentration, irrespective of the velocity of flow of the lamina. This prolongs the time course of the curve and depresses the peak concentration. This mean concentration recording should be contrasted with mean flux recording, as obtained by intermittent sampling techniques, where the rate at which different laminae of flow deliver dye into a series of sampling tubes depends on the product of concentration and flow velocity. In this case, the mean concentration value is weighted in favour of the axial, more rapid laminae, and will produce displacement of the dye concentration curve in the direction of increasing time, but with less distortion of its shape (Sheppard et al., 1959).

The second source of distortion is that of time-average sampling instead of volume-average sampling (Visscher and Johnson, 1953; Stow, 1954). Short of a sampling system which produces flow rates proportional to the cyclic flow changes at its tip, which seems mechanically insurmountable, this error cannot be overcome. Experimental studies, however, suggest that the error introduced by this problem in the calculation of cardiac output is insignificant (Bassingthwaighte et al., 1962).

The Calibration Method

The method of calibration was described in an earlier section. It will be recalled that volumes of dye, not exceeding 0.188 ml., were added to 25 ml. aliquots of arterial blood in making up the range of calibration dye-blood samples. Miller et al. (1962) believe that pipetting such small volumes introduces a definite source of error. The linearity of the calibrations obtained in this study suggest otherwise (figure 3), unless the error was a systematic one giving a calibration factor constantly too high or too low. Since this would cause the dye results for cardiac output to be consistently too great or too small, one would expect poor agreement with the Fick values for cardiac output, which in fact is not the case, as will be demonstrated in the results of the dye-Fick comparison.

To assess the accuracy of the volumes of dye solution pipetted into 25 ml. aliquots of blood, volumes of dye solution covering the range of volumes pipetted for calibration were pipetted into dry weighed flasks which were then reweighed. Table 4 gives the results of the estimated volumes as checked by weighing.

The standard error of a single observation was found to be 0.11 per cent. and one S.D. 0.4 per cent..

Sinclair et al. (1961) have recently described a method of calibration very similar to that used in the present study. They demonstrated the errors introduced by transparent solutions in monochromatic densitometry, and for that reason have rejected the previous methods, which employ large volumes of saline in the dilution process involved in making up calibration

TABLE 4

Volume of dye solution pipetted (ml.)	Actual volume by weight (g.)	Percentage error
0.025	0.0249	-0.36
0.037	0.0372	+0.54
0.050	0.0507	+1.40
0.062	0.0629	+1.45
0.075	0.0753	+0.40
0.087	0.0874	+0.46
0.100	0.1001	+0.10
0.112	0.1116	-0.36
0.125	0.1256	+0.48
0.137	0.1367	-0.22
0.150	0.1504	+0.27
0.162	0.1633	+0.80
0.175	0.1759	+0.51
0.188	0.1895	+0.80

samples (Nicholson and Wood, 1951). Such methods were perfectly adequate for use in the dichromatic converted oximeter which Wood's group used, but they have been perpetuated into monochromatic densitometry where their potential errors could be serious. Nevertheless, the present method is less time-consuming, and could equally well be applied to dichromatic instruments.

In the present study a shorter, wider catheter than the one used in the patient was employed for the calibration. At the volume flow ratio used for recording dye-dilution curves the withdrawal rate is as high as is physically possible for the combination of catheter, cuvette, connecting tubing and tap volumes used. With the catheter tip in the aorta, there is an average mean arterial pressure head of approximately 90 mm. Hg., which serves to lower the pressure gradient across the withdrawal system, and makes an adequate withdrawal rate of 38.0 ml./min. possible. Once outisde the body, however, this pressure head is lost, as during calibration, and this loss is sufficient to cause cavitation in the pump syringe, and an unstable withdrawal rate. As the withdrawal rate falls off, so the calibration trace drifts. To keep the withdrawal rate absolutely constant, an essential requisite for both dye curve inscription and calibration, as was discussed in a previous section, a larger bore, shorter catheter was therefore used during the calibration.

There are two other available principles of calibration when indocyanine green is used. One of these is the so-called "integral sampling" calibration method of McNeely and Gravallese (1955), and

Emanuel, Lacy and Newman (1957). It entails the channelling off, during the inscription of a dye curve, of part or all of the dyed blood after its traversal of the cuvette via a system of taps. This dyed blood specimen contains the average concentration of dye for a marked portion of the actual dye curve. After mixing and centrifugation, the plasma dye concentration is determined spectrophotometrically to find the mean dye concentration in plasma, from which the mean dye concentration in blood can be derived, using the haematocrit. The method is ingenious, but potential errors include the possible change in withdrawal rate when the tap redirects the blood flow into an unprimed syringe for collection of the "integral sample", the error of any deadspace between cuvette centre and tap assembly, and the error of not absolutely accurate time markers on the dye curve, indicating exactly which two points on the trace designate the collected section of the dyed blood. Considering that the blood loss involved is no less for a single calibration point as obtained in the present system, and the margin of error is greater with this method, it seems to offer no particular advantage.

A more recent method of dynamic calibration has been described by Sparling, Mook, Nieveen, v.d. Slikke and Zijlstra (1960), and assessed in model studies by Emanuel and Norman (1963). It has the advantage of avoiding any form of chemical or spectrophotometric analysis, and of any blood loss. The area of a dilution curve obtained from a patient is compared with that derived from a small calibration system built into the sampling line, which is simply a site at which a dye bolus can be injected between sampling catheter tip and cuvette. This latter system allows a dye curve to be fashioned within the sampling system itself. If the areas of the dilution curve from both patient and calibration system are the same, the flowrate in the patient (Q) is related to the flowrate in the calibration system (Qc) as the quantity of dye injected into the patient (I) is related to the quantity of dye injected into the calibration system (Ic), i.e. Q : Qc as I : Ic, or $Q = \frac{I \times Qc}{Ic} \times \frac{Ac}{A}$ or $Q = \frac{I \times Qc \times Ac}{Ic \times A}$. The equation can be solved for Q, as I, Ic, A, Ac and Qc can all be measured.

Ingenious though it is, the method has disadvantages if used in the present dye-dilution apparatus. It would require an injection and mixing system incorporated between sampling catheter and cuvette, which would

greatly increase hydraulic curve distortion even if the calibration system could be excluded during the inscription of dye curves, because of the added volume of the necessary taps. It would have to be designed, not only to provide adequate mixing between injection site and cuvette, but would require a volume of at least 1.26 ml. to allow adequate time for stabilisation of the spectral transmission of the dyed blood at a withdrawal rate of 0.63 ml./sec., assuming that stabilisation of the indocyanine green and blood mixture spectral transmission takes place within two seconds. If the procedure were performed after the investigation "in vitro", it would have no advantage over the present calibration method, as blood removal would be necessary, and more variables per calibration point must be measured in Sparling's method.

It would naturally be preferable to dispense with calibration for each individual subject entirely. Nicholson and Wood (1951) originally tried to relate the calibration factor to haemoglobin concentration, but found that there was no correlation. Falholt and Kaiser (1955) claimed to have an instrument which did not require calibration for each patient, due to compensation in the green band of the spectrum for non-specific optical density factors. Sekelj et al. (1958) derived a calibration factor by comparing their dye-Fick results, which theyincorporated in their formula for cardiac output, to serve as a calibration for all subjects. Their results were not at all satisfactory, as this is a gross over-simplification of the problem. The reflection, transmission and refraction of incident light are affected by numerous factors (Lothin and

Lewis, 1956), which include the shape of the erythrocytes (Nakamura and Amada, 1957), the size of the erythrocytes (Orskov, 1934), plasma osmolarity (Read, Johnson, Vick and Meyer, 1960), temperature (Jacobs, Glassman and Parpart, 1936; Pappenheimer, 1941), pH (Brown, 1956), and the rate of flow of blood through the cuvette (Kramer, 1935, 1950; Pappenheimer, 1941; Opitz, 1948; Wood, 1950; Zijlstra, 1953). Individual calibration therefore seems essential in monochromatic densitometry, and the method described appears the most satisfactory one available.

THE COMPARISON OF THE DYE-DILUTION METHOD WITH THE FICK METHOD

As was mentioned in a previous section, the direct Fick method is generally regarded as the most reliable available method of determining cardiac output. It was also pointed out, however, that the exigencies of the method limit its use. It remains, nevertheless, the best yardstick by which to check the accuracy of the dye-dilution method described, but, by its own very limitations, any such comparison requires rigorously defined conditions in order to guarantee that the Fick method itself is not being employed in circumstances in which its own accuracy is compromised. The Fick principle is subject to several errors which will be discussed in a later section; many of them are readily reduced by sensible application of the technique.

As was discussed earlier, many previous dye-Fick comparisons were open to two serious criticisms: technical inadequacies, and poor design of the comparison. The present study was designed to reduce such errors to a minimum, and to yield certain other information at the same time. The points to be demonstrated are as follows:

i) In a heterogeneous group of 25 subjects, the cardiac output determined by the dye-dilution method described did not differ from that obtained by a simultaneous Fick determination. This was assessed during five consecutive four minute periods of rest during which everything possible was done to ensure the utmost stability of each subject in order that the Fick estimation would attain its greatest accuracy.

ii) Under less stable conditions of "steady-state" exercise a similar comparison was performed in most of the 25 subjects at one or two levels of exercise, and also in single resting observations before the period of exercise. This section of the study was intended to show that the accuracy of the agreement between the two methods was reduced in such circumstances, and would account to some extent for some of the less satisfactory results of previous comparisons.

iii) Injection into the pulmonary artery, instead of the right atrium, did not alter the accuracy of the dye-dilution method when compared with the Fick method in an absolutely steady state.

iv) A more basal state is best achieved following a short period of light to moderate exercise. This will be demonstrated by a comparison of the cardiac output prior to the exercise period with the first post-exercise value.

v) The dye-dilution method of cardiac output gives closely reproducible results when repeated at one or two minute intervals provided that the subject under investigation is in a steady state, as assessed by a steady heart rate.

Clinical Data

The comparison between the dye-dilution and Fick methods of measuring cardiac output was made in 25 subjects of varying ages, some with heart disease and some without. Details of each patient are as follows:

A.C. (Male), Age: 24 yr., Ht.: 1.68 m., Wt.: 81.2 kg., S.A.: 1.91 sq.m.
Normal subject.
X-ray chest: normal.
E.C.G.: normal.

R.B. (Female), Age: 36 yr., Ht.: 1.57 m., Wt.: 58.0 kg., S.A.: 1.57 sq.m.
Normal subject.
X-ray chest: normal.
E.C.G.: left ventricular strain.

J.R. (Male), Age: 48 yr., Ht.: 1.68 m., Wt.: 74.2 kg., S.A.: 1.83 sq.m.
Normal subject.
X-ray chest: normal
E.C.G.: normal.

L.F. (Male), Age: 20 yr., Ht.: 1.80 m., Wt.: 109.0 kg., S.A.: 2.27 sq.m.
Obesity.
X-ray chest: normal.
E.C.G.: normal.

R.G. (Male), Age: 46 yr., Ht.: 1.65 m., Wt.: 68.0 kg., S.A.: 1.74 sq.m.
Duodenal ulcer.
X-ray chest: normal.
E.C.G.: questionable left ventricular hypertrophy.

C.M. (Female), Age: 56 yr., Ht.: 1.62 m., Wt.: 60.9 kg., S.A.: 1.63 sq.m.

Intermittent claudication.

X-ray chest: normal.

E.C.G.: slight left ventricular strain.

C.McK. (Male), Age: 51 yr., Ht.: 1.52 m., Wt.: 42.8 kg., S.A.: 1.34 sq.m.

Convalescent pneumonia.

X-ray chest: normal.

E.C.G.: normal.

C.J. (Male), Age: 57 yr., Ht.: 1.64 m., Wt.: 60.6 kg., S.A.: 1.64 sq.m.

Chronic bronchitis.

X-ray chest: normal.

E.C.G.: normal.

J.N. (Male), Age: 37 yr., Ht.: 1.57 m., Wt.: 58.0 kg., S.A.: 1.57 sq.m.

Chronic bronchitis.

X-ray chest: normal.

E.C.G.: normal.

J.S. (Male), Age: 54 yr., Ht.: 1.57 m., Wt.: 46.3 kg., S.A.: 1.43 sq.m.

Emphysema.

X-ray chest: normal.

E.C.G.: normal.

C.McQ. (Female), Age: 14 yr., Ht.: 1.60 m., Wt.: 55.8 kg., S.A.: 1.56 sq.m.

Severe anaemia (Hb. 6.3 g.%; P.C.V. 23%).

X-ray chest: normal.

E.C.G.: not done.

M.C. (Female), Age: 61 yr., Ht.: 1.48 m., Wt.: 68.3 kg., S.A.: 1.61 sq.m.

Essential hypertension (B.P. 210/115).

X-ray chest: some cardiac enlargement which is mainly left ventricular.

E.C.G.: left ventricular strain.

R.A. (Male), Age: 38 yr., Ht.: 1.78 m., Wt.: 74.5 kg., S.A.: 1.91 sq.m.

Essential hypertension (B.P. 190/110); angina pectoris; duodenal ulcer.

X-ray Chest: normal.

E.C.G.: normal.

B.R. (Female), Age: 36 yr., Ht.: 1.57 m., Wt.: 65.8 kg., S.A.: 1.65 sq.m.

Essential hypertension (B.P. 180/115); myxoedema.

X-ray chest: left ventricular hypertrophy.

E.C.G.: left ventricular strain.

M.S. (Female), Age: 42 yr., Ht.: 1.67 m., Wt.: 76.0 kg., S.A.: 1.84 sq.m.

Essential hypertension (B.P. 160/110).

X-ray chest: left ventricular hypertrophy.

E.C.G.: normal.

C.C. (Male), Age: 42 yr., Ht.: 1.66 m., Wt.: 71.0 kg., S.A.: 1.78 sq.m.

Renal artery stenosis with hypertension (B.P. 150/105); old hemiplegia.

X-ray chest: normal.

E.C.G.: normal.

G.C. (Male), Age: 32 yr., Ht.: 1.78 m., Wt.: 78.5 kg., S.A.: 1.95 sq.m.

Pulmonary hypertension - ? left ventricular myocarditis; ? left atrial myxoma.

X-ray chest: normal.

E.C.G.: normal.

O.McN. (Male), Age: 58 yr., Ht.: 1.65 m., Wt.: 73.5 kg., S.A.: 1.80 sq.m.
Essential hypertension (B.P. 190/120); atrial fibrillation.
X-ray chest: left ventricular and left atrial enlargement.
E.C.G.: atrial fibrillation.

G.T. (Male), Age: 63 yr., Ht.: 1.63 m., Wt.: 58.5 kg., S.A.: 1.61 sq.m.
Chronic bronchitis and emphysema; atrial fibrillation; previous right-sided cardiac failure.
X-ray chest: some generalised cardiac enlargement.
E.C.G.: atrial fibrillation.

J.C. (Male), Age: 21 yr., Ht.: 1.70 m., Wt.: 56.8 kg., S.A.: 1.64 sq.m.
Aortic stenosis; mitral stenosis.
X-ray chest: left ventricular hypertrophy.
E.C.G.: left atrial hypertrophy.

E.S. (Female), Age: 52 yr., Ht.: 1.55 m., Wt.: 61.7 kg., S.A.: 1.59 sq.m.
Aortic stenosis; mitral stenosis (operative findings: shrunken left atrium, mitral valve admitted tip of index finger, no incompetence); atrial fibrillation.
X-ray chest: enlarged left atrium.
E.C.G.: atrial fibrillation.

J.G. (Female), Age: 56 yr., Ht.: 1.52 m., Wt.: 48.0 kg., S.A.: 1.42 sq.m.
Mitral stenosis; atrial fibrillation.
X-ray chest: generalised cardiac enlargement.
E.C.G: atrial fibrillation, ? left ventricular hypertrophy.

A.G. (Female), Age: 46 yr., Ht.: 1.55 m., Wt.: 71.4 kg., S.A.: 1.70 sq.m.
Mitral stenosis; atrial fibrillation.
X-ray chest: moderate left atrial enlargement, right ventricular enlargement.
E.C.G.: atrial fibrillation.

E.R. (Female), Age: 57 yr., Ht.: 1.52 m., Wt.: 54.0 kg., S.A.: 1.49 sq.m.
Atrial fibrillation; mitral stenosis (operative findings: thick left atrium, mitral valve admitted tip of index finger, no incompetence).
X-ray chest: left atrial and left ventricular enlargement.
E.C.G.: atrial fibrillation, left ventricular hypertrophy.

E.T. (Female), Age: 45 yr., Ht.: 1.53 m., Wt.: 52.9 kg., S.A.: 1.48 sq.m.
Mitral stenosis; atrial fibrillation.
X-ray chest: some generalised cardiac enlargement with predominance of the left ventricle.
E.C.G.: right ventricular hypertrophy.

Plan of Investigation

A few days prior to the investigation, each subject was introduced to the laboratory and its staff in order to familiarise him with the surroundings, and the intended procedure was explained in full. He exercised at several levels in order to choose a level which he could maintain steadily with comfort, and to reassure him of his own capability.

The investigation was carried out in the supine position at least three hours after a light meal. After the catheters were positioned, approximately twenty minutes were allowed for the subject to settle down before the first definitive measurements of cardiac output were made.

During this twenty minute period, 100 ml. of arterial blood were taken off for calibration of the dye-dilution apparatus at the end of the study, as described earlier. Control samples were also taken for later assessment of thrombin time by the toluidine-blue thrombin clotting time method. Trial dye curves were performed to assess the optimum sensitivity for recording and the most suitable paper speed adjustment. The trial curves were timed in order to ascertain whether one curve per minute, or one curve every other minute only, could be performed in the subject under investigation at rest, according to his speed of circulation. During exercise, one dye curve per minute was possible in all subjects however.

In most subjects the procedure, illustrated diagramatically in figure 17, was as follows. A four minute period of rest was followed by two consecutive six minute periods of cycling at different exercise levels on a constant-speed, variable-load ergometer. Definitive measurements were made during the entire four minute rest period, and the last two minutes only of each six minute exercise period. The subject then rested for twenty minutes before six consecutive four minute recovery periods began. These were as rapidly consecutive as was compatible with the expired air sampling necessary between each, and were not more than five minutes apart. After five such periods, during which the dye was injected into the right atrium, in the sixth and final recovery period it was injected into the pulmonary artery.

The five recovery periods followed the plan illustrated in figure 17 in all subjects, but in some subjects the initial rest period and or the

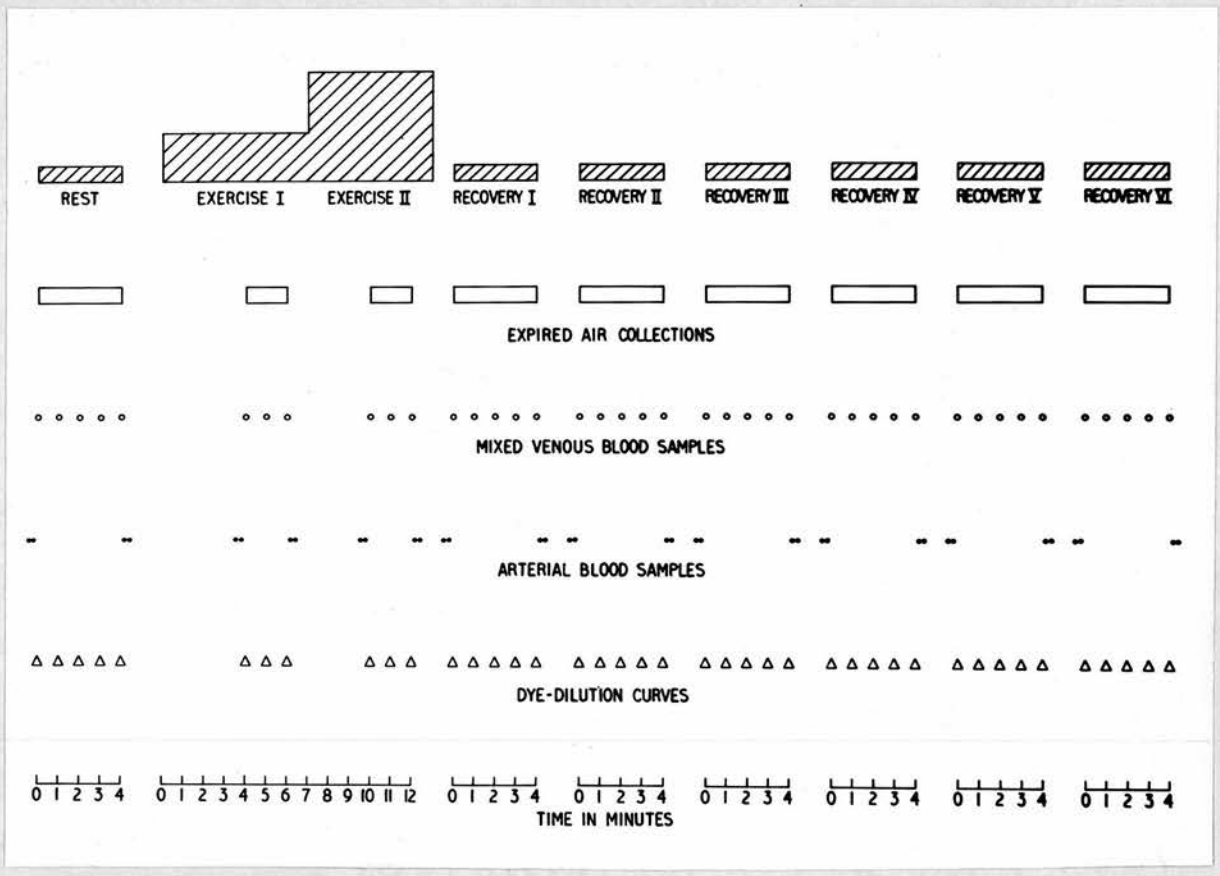

Figure 17: Plan of dye-Fick comparison

sixth recovery periods were omitted. In some subjects a full twelve minutes exercise were not possible, and only one exercise period was obtained. A few subjects could not tolerate even one full six minute period, and in these subjects exercise was allowed for as long as was comfortable without measuring cardiac output, and the recovery periods then continued as usual.

Dye injections were made so that the peak of each curve coincided with each minute, or every other minute, of the Fick expired air collection. Dye curves, arterial and mixed venous blood samples, and expired air collections were related to each other, as illustrated in figure 17.

Before removal of the catheters at the end of the investigation, any diagnostic pressure readings required were obtained. Blood was taken to check the thrombin time with the earlier control value. If the result was satisfactory, the catheters were removed and the patient returned to the ward.

Laboratory Techniques

The laboratory air temperature was controlled at 20 - 23 ^{o}C (68 - 73^{o}F) in all studies; the relative humidity varied between 45 and 73 per cent., but never more than 3 per cent. during the course of a single study.

A 9F, 125 cm. triple-lumen catheter (United States Catheter & Instrument Corp.) was introduced under local anaesthesia (two per cent. Duncaine) by a cut-down procedure, into the median basilic vein. It was positioned under radiological screening control so that its distal and middle lumens were in the pulmonary artery, and its proximal lumen in the

right atrium. Pulmonary arterial and right atrial pressures were transduced by two Statham P23Db strain gauge manometers (figure 18). The catheter orifices were 15 cm. apart, and the position of each orifice was confirmed by checking the pressure waveforms on the oscilloscope trace. The three lumens were kept patent by continuous drips of heparinized saline (ten units heparin per ml.).

Another catheter was then introduced into the brachial artery of the same or opposite arm under local anaesthesia. This 4F, 55 cm. Portex nylon catheter with a Luer mount was of internal diameter 1.00 mm., and external diameter 1.34 mm., and was introduced percutaneously by a modified Seldinger technique over 0.8 mm. nylon, threaded through a Riley arterial needle. The catheter was advanced until all but six to eight centimetres were intravascular, and the waveform was checked to ensure that the tip lay in the aortic root. Aortic blood pressure was transduced by a Statham P23Db strain gauge manometer with a predetermined critical hydraulic damping (Taylor, Sutherland, Hutchison, Kidd, Robertson, Kennelly and Donald, 1962). A continuous electrocardiograph trace (Lead CR6) was recorded throughout the procedure on both the oscilloscope and the U.V. recorder trace.

Expired air was collected in a Tissot spirometer, and the collected volume always exceeded the minimum dilution factor of 21 litres. Before each Fick period, expired air was collected and was discarded immediately prior to the definitive expired air collection. Ventilation volumes were measurable to an accuracy of 0.3 per cent. on the spirometer trace.

Expired air samples were taken in mercury-filled tonometers and were

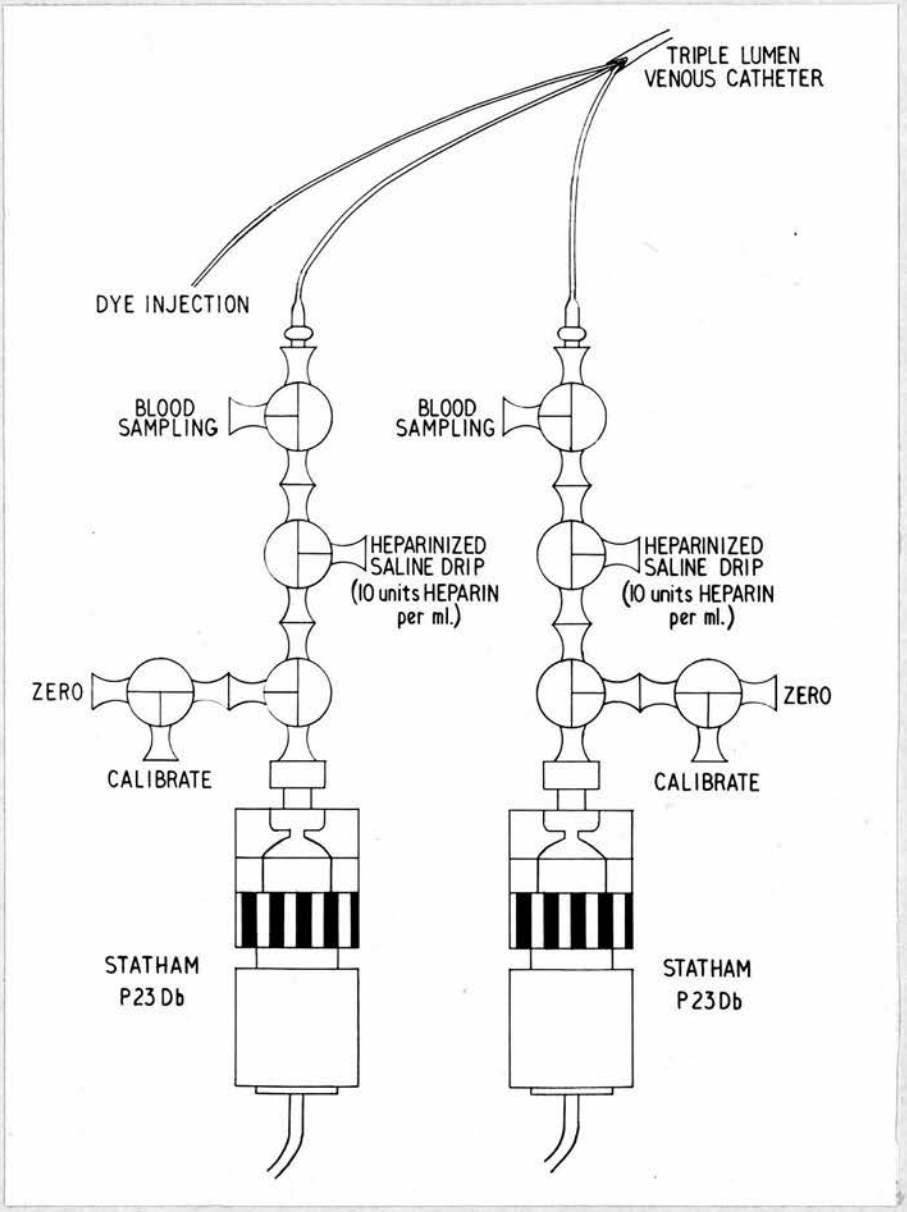

Figure 18: Venous pressure and sampling tap and drip assembly

analysed in duplicate on a Scholander gas analyser (Scholander, 1947). Analysis of room air was carried out in each case immediately prior to commencement of the analysis of the expired air samples, and the room air mean oxygen concentration obtained was 20.91 per cent. (S.D. 0.046 per cent). Duplicate analyses of oxygen and carbon dioxide concentrations agreed to within 0.06 per cent.

Aortic and pulmonary arterial blood samples were taken in heparinized syringes. Mixed venous blood samples were withdrawn over a period of 15 - 20 seconds, coinciding with each minute of the Fick period. Arterial blood samples were withdrawn over a period of 5 - 10 seconds at the beginning and end of each Fick period. Their oxygen saturations were estimated in duplicate using a Brinkman haemoreflector, and duplicates were required to agree to within 0.5 per cent. saturation. The manufacturers of the instrument recommended an individual calibration line to be constructed for each subject from oxygenated, reduced, and lower-reduced specimens of the subject's blood, and plotting the galvanometer deflections obtained from these specially prepared blood samples. This empirical method was found to be inaccurate at lower oxygen saturations, and the following procedure was adopted. Numerous blood samples from different subjects were tonometered at varying oxygen tensions to obtain specimens over a wide range of oxygen saturations. Oxygen saturation values of these samples were obtained by a manometric technique (van Slyke and Neill, 1924), and galvanometer readings from these same samples were recorded by the haemoreflector. The relationship between the Brinkman galvanometer

deflections and the van Slyke saturations is shown in figure 19. When the individual calibration lines from all these bloods were also plotted, they were found to show a considerable scatter, especially at lower oxygen saturation levels. A regression line was calculated for the relationship between oxygen saturation, determined by the van Slyke method, and the haemoreflector galvanometer deflections (figure 19). The equation for the line is: $y = 14.9 + 2.12x - 0.00821\,x^2$, where y represents percentage saturation, and $x = 100 \left[\log \frac{(\text{galvanometer deflection})}{10} - 0.5 \right]$. The resultant relation between the van Slyke and haemoreflector values for oxygen saturation using this equation is illustrated in figure 20. It is apparent that, despite this improvement, mixed venous oxygen saturations in the lower ranges can be read with confidence to the nearest one per cent. only, although arterial saturations are accurate to 0.1 per cent.

The oxygen-carrying capacity of the blood was measured by a standard spectrophotometric technique, using a 0.04 per cent. solution of ammonia as the diluting agent (Wintrobe, 1961), on a Unicam SP 600 which was calibrated against the van Slyke method ($r = 0.986$) (figure 21). The oxygen-carrying capacity, expressed in volumes per cent., was obtained from the regression formula: oxygen-carrying capacity = 39.49 x optical density + 0.767. The standard error of a van Slyke estimate was estimated to be 0.490, and that of a spectrophotometric estimate 0.012.

Calculation of the Fick cardiac output was performed as follows. A previously ascertained factor was used to convert the spirometer trace deflection into ventilation volume in litres, which was then calculated

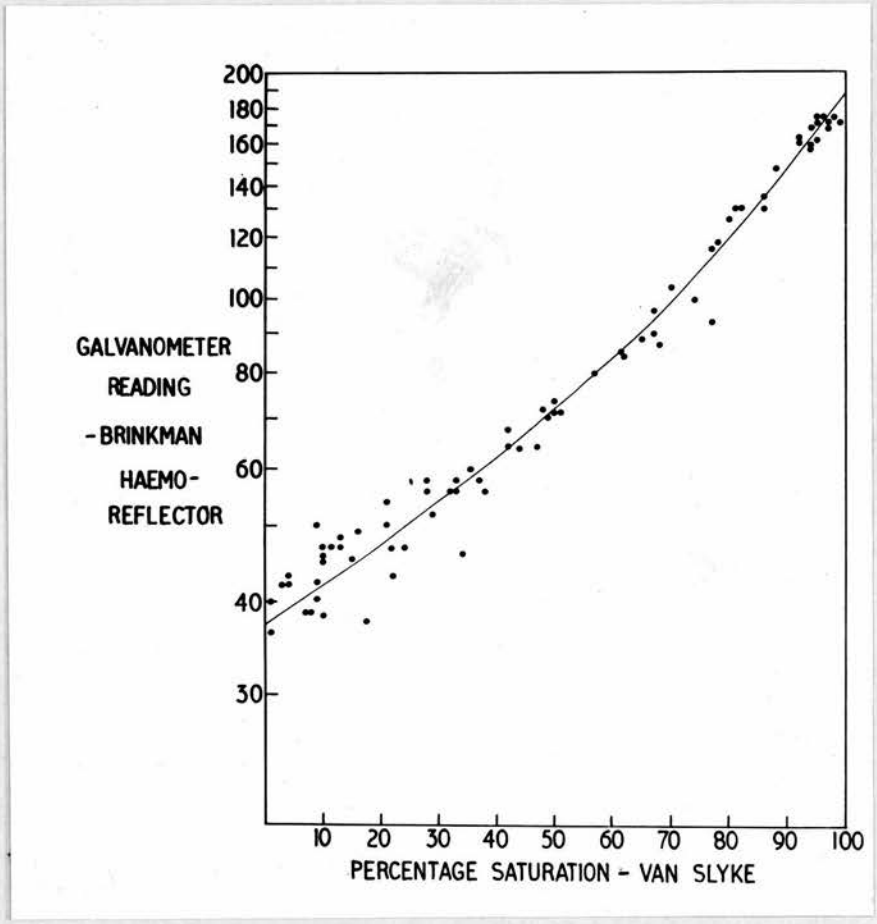

Figure 19: Comparison of van Slyke saturation values and Brinkman haemo-reflector galvanometer readings

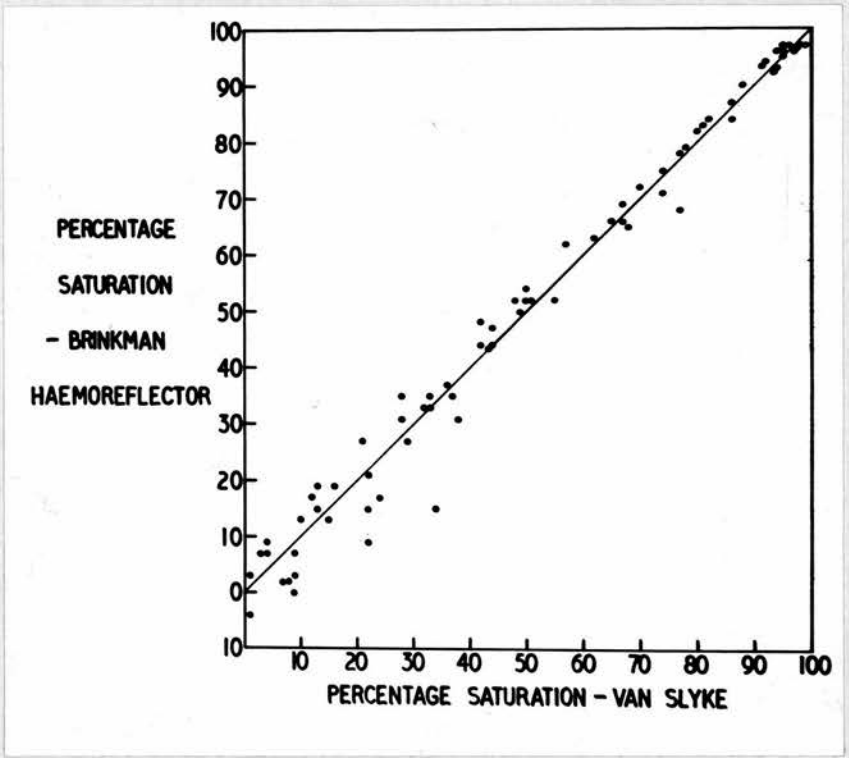

Figure 20: Comparison of van Slyke and Brinkman haemoreflector oxygen saturation values using corrected calibration

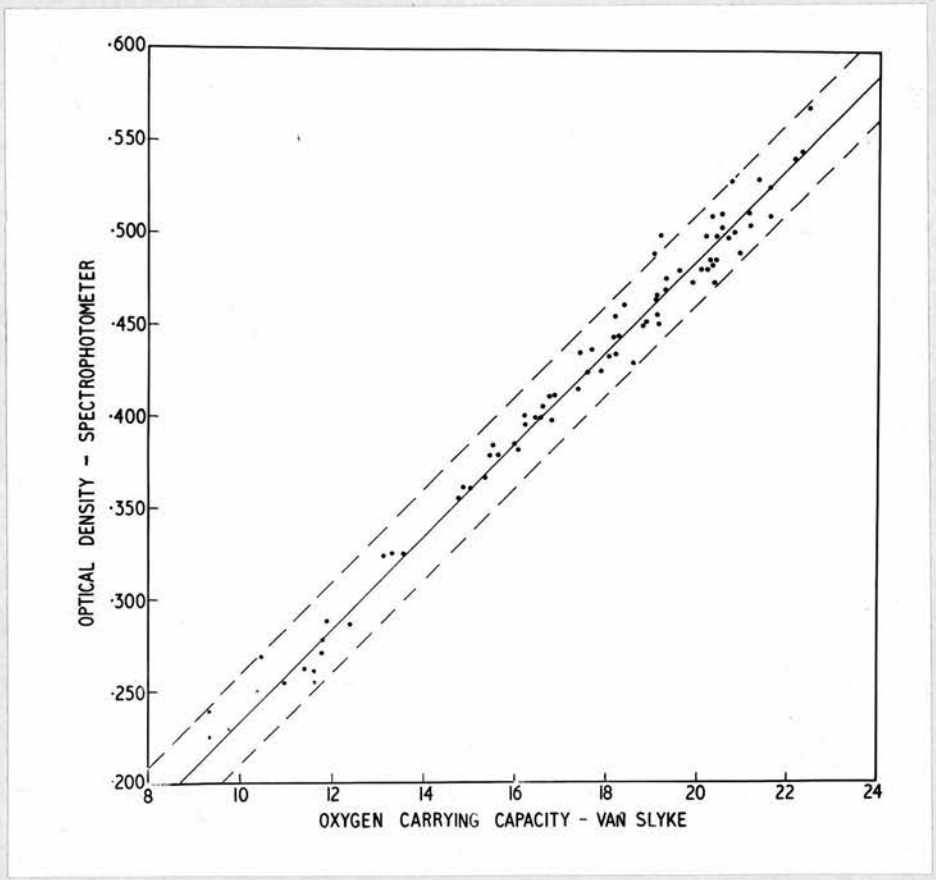

Figure 21: Comparison of oxygen carrying capacity values (van Slyke) and Unicam SP 600 optical density readings

in litres per minute. The barometric pressure, corrected for brass scale and mercury column distortion by ambient temperature, and the temperature of the expired air sample, were used to correct this volume at S.T.P.D. to B.T.P.S. (Carpenter, 1948). The value for expired nitrogen was derived from the Scholander analysis of expired air for oxygen and carbon dioxide. Inspired carbon dioxide was accepted as 0.03 per cent., and inspired oxygen as 20.91 per cent. (the average Scholander reading). Correction of the above inspired oxygen concentration value was carried out in the conventional way (Carpenter, 1948). Oxygen extraction, thus derived, was multiplied by the ventilation volume in litres per minute (S.T.P.D.) to obtain the oxygen uptake in litres per minute.

The values of the two arterial oxygen saturation readings were averaged and subtracted to give the arteriovenous oxygen saturation difference. This value was multiplied by the mean value of the two arterial oxygen-carrying capacity samples taken at the beginning and end of the period of expired air collection to yield the arteriovenous oxygen content difference. The cardiac output was then calculated according to the formula: Fick cardiac output = $\frac{\text{oxygen uptake (ml./min.)}}{\text{A-V oxygen content difference (ml./l.)}}$.

Dye curves were inscribed using the sampling-detecting-recording system already described. The dye-dilution apparatus was calibrated with the subject's own blood after each study, and the cardiac outputs calculated as described earlier.

RESULTS

RESULTS OF THE DYE-FICK COMPARISON AND THEIR ANALYSIS

Cardiac outputs by the dye-dilution and Fick methods were calculated according to the methods already described. Appearance times, mean transit times and peak concentrations were corrected for distortion by the sampling system according to the regression equations of Milnor and Jose (1960) described earlier. Central blood volumes were calculated according to the formula: $V = \frac{Q \cdot Tm}{60}$, using the corrected mean transit times. Tables 5 - 29 contain the Fick and dye-dilution results during the five four-minute recovery periods, Table 30 the results during the initial pre-exercise rest period, Table 31 the results during exercise, and Table 32 the results during the sixth recovery period where dye was injected into the pulmonary artery.

Table 33 contains a statistical analysis of previous dye-Fick comparisons in which the data published made such an analysis possible. Also on Table 33 is the statistical analysis of the present dye-Fick comparison, divided into four groups (Tables 5 - 29, 30, 31, and 32). The statistical methods used are described in the Appendix.

DISCUSSION

DISCUSSION OF THE RESULTS OF THE DYE-FICK COMPARISON

The Comparison of the Dye-Dilution and Fick Values for Cardiac Output during the five Consecutive Post-Exercise Recovery Periods (Tables 5-29; Figure 22)

It has been repeatedly stressed that the results of the two methods can only be compared with confidence when the Fick method attains its greatest accuracy. The study was therefore planned so that the subjects would be as stable as possible during the five four-minute recovery periods, and the formal comparison was made at this time. Table 33 shows the very high degree of correlation obtained ($r = 0.991$), which is better than that obtained in any previous reported study. The regression coefficient ($b = 0.983$) does not differ significantly from the line of identity ($0.40 > P > 0.30$), showing therefore that no systematic error is present. The only previous study showing comparable accuracy is that of Kopelman and Lee (1951). Their results were truly remarkable since they were handicapped by the undoubtedly inferior technique of intermittent sampling, and carried out only one dye-dilution curve per Fick estimate.

It is not surprising that the results of the present comparison do not show absolute agreement although they were performed simultaneously, unlike most previous studies. Individual values for cardiac outputs by the dye technique vary quite considerably from minute-to-minute within a single Fick period in many apparently stable subjects. This points to the far greater discrepancy between Fick and dye values which would result if only one of the individual dye values were compared with the Fick value, as was the case in all but one of the previous studies (Falholt and Fabricius, 1956).

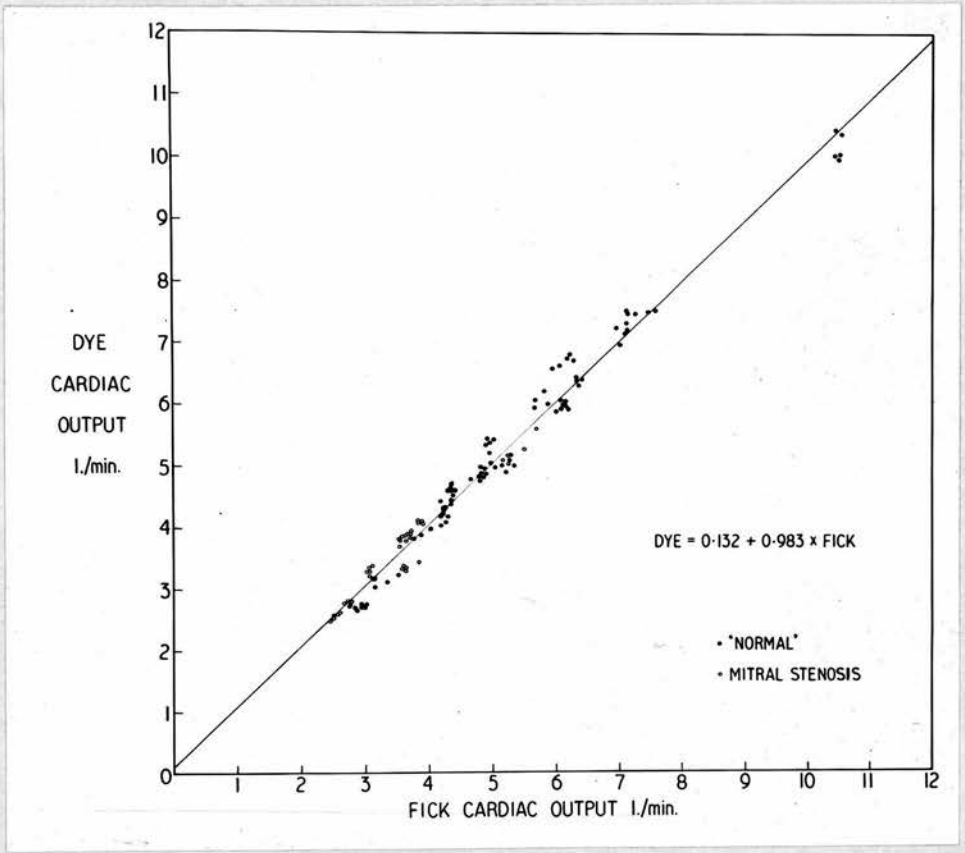

Figure 22: Comparison of simultaneous Fick and dye methods for cardiac output during post-exercise recovery

The use of the mean of three or five dye-dilution cardiac outputs in comparison with each Fick period obviously reduces this discrepancy, but can only be an incomplete solution to the inevitable error which must result from comparing two methods which measure flow over such different time periods. The present study, limited though it is by this unavoidable discrepancy between the two methods, demonstrates the accuracy of the dye-dilution method described. The unsatisfactory results of many previous studies are almost certainly due to failure to attend to detail in the design of the experiment and the methods used, especially with regard to the Fick method, and not due to any inherent shortcoming of the dye-dilution principle.

The Comparison of the Dye-Dilution and Fick Values for Cardiac Output during the Single Pre-Exercise Rest Period (Table 30; Figure 23), and the Exercise Periods (Table 31; Figure 24)

Evidence will be presented that the cardiac output during the initial rest period is not that of as basal a state as that achieved during the first recovery period 20 minutes after cessation of exercise. It might therefore be expected that a subject would possibly not achieve as steady a state during this initial rest period as following exercise, and that slight errors in the Fick method might result. Contrary to expectations, however, the results of the comparison during this pre-exercise rest period show a high degree of correlation ($r = 0.980$) and the regression coefficient ($b = 0.907$) does not differ significantly from the line of identity ($0.30 > P > 0.20$).

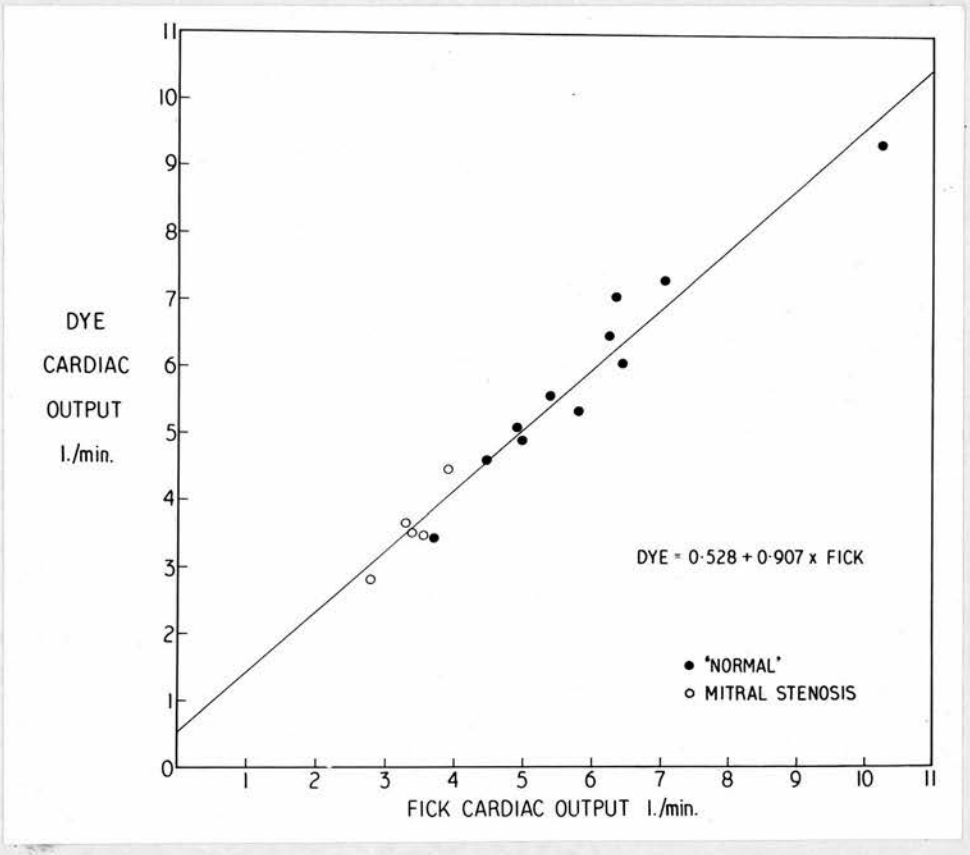

Figure 23: Comparison of simultaneous Fick and dye methods for cardiac output during pre-exercise rest

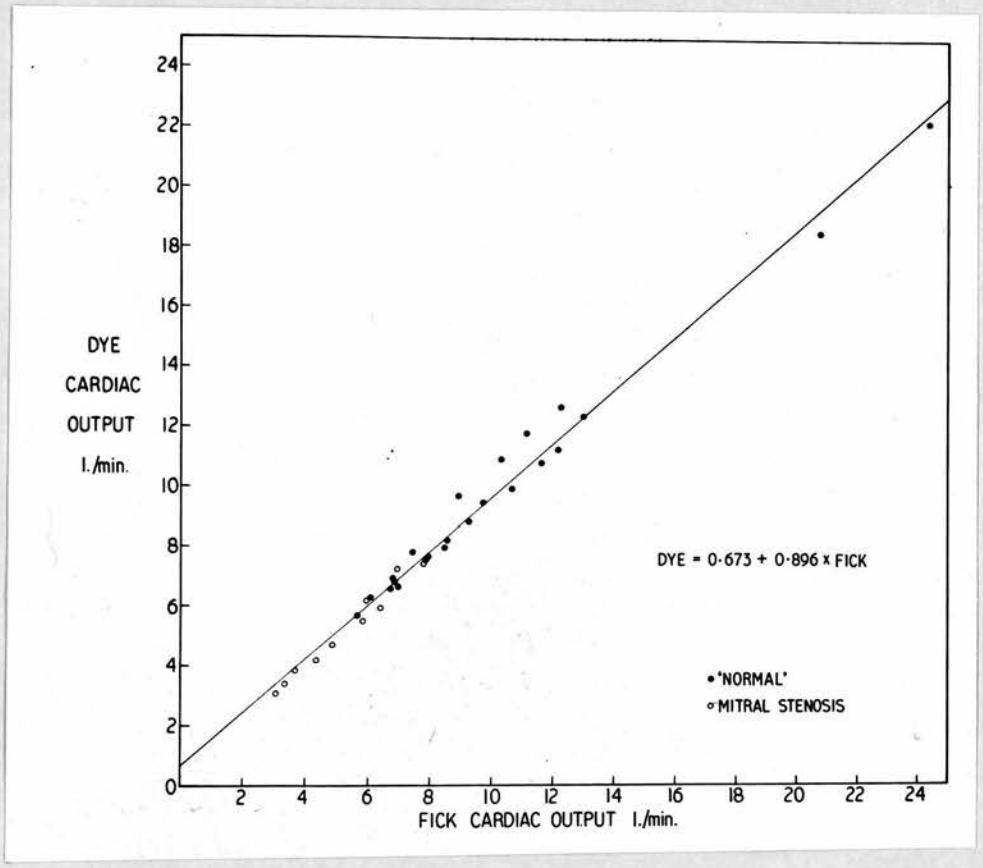

Figure 24: Comparison of simultaneous Fick and dye methods for cardiac output during steady-state exercise

During exercise the Fick method is liable to several sources of error which will be described in a later section. Donald, Bishop, Cumming and Wade (1955) claim that a steady-state is reached after the first few minutes of exercise and that the Fick method is valid in such circumstances. The present study shows a high degree of correlation ($r = 0.994$) during exercise, but the regression coefficient ($b = 0.896$) differs significantly from the line of identity ($P < 0.001$). The dye cardiac output values were therefore systematically low in the higher ranges of cardiac output or the Fick values were systematically high. To try to account for this systematic error the various component measurements in both methods will be considered.

In the dye-dilution method, both the weight of dye injected and the calibration factor should not change during exercise. The measurement of the area of the dye curve should be no less accurate because the adjustment of the recorder paper speed and sensitivity maintained the curve geometry relatively constant irrespective of the cardiac output.

The ventilation per minute is greater during exercise, but the period of measurement is shorter (two minutes during exercise compared to four minutes at rest), and so the volumes measured are not dissimilar. The value for oxygen extraction depends on the Scholander gas analysis, and the accuracy of this method is not reduced when exercise expired air samples are analysed. The value for the oxygen carrying capacity of the blood changes very slightly during exercise, but within a range accurately measurable by the method employed. Although the error of reading the

oxygen saturation of mixed venous blood samples increases as their saturation decreases during exercise, the error is not a systematic one (Figure 20).

Since the methods used in the estimation of both dye and Fick values do not apparently account for the systematic error, it may be that the subjects did not achieve an absolutely steady state during exercise. In their determinations of the cardiac output by the Fick method during steady-state exercise, Donald et al. (1955) assessed the errors in the estimation of cardiac output and oxygen consumption. The largest of these errors which could be invoked to explain an erroneously high Fick value during exercise was due to the overestimation of oxygen uptake due to a change in alveolar oxygen tensions. This error was no larger than one per cent., which could hardly explain the error in the present study during exercise.

Evidence will be presented that the dye values for cardiac output are accurately reproducible in closely consecutive readings. The variations in the dye values within each Fick period can therefore be assumed to give a true representation of changes in flow during the period of measurement of flow by the Fick method, and Table 31 gives an idea of the variation of the A-V oxygen saturation difference during each exercise Fick period. Using the method of Visscher and Johnson (1953) an assessment of the error due to both flow and A-V oxygen saturation differences changing together during a Fick period can be obtained. Both measurements will be seen to vary over a very narrow range, quite insufficient to account for a systematic error in the Fick during exercise of the order observed. No grounds, however, can be offered for

questioning the validity of the dye-dilution method during exercise.

The discrepancy between the results of the two methods remains unexplained therefore. Whatever the reason for the discrepancy, it may be that the same factor accounted to some extent for the poor results of certain previous studies where exercise studies were included in the same comparison as the resting studies (Hamilton et al., 1948; Eliasch et al., 1954; Shepherd et al., 1955; Miller et al.,1962).

The Comparison of the Dye-Dilution and Fick Values for Cardiac Output during the Sixth Recovery Period with Dye Injection into the Pulmonary Artery (Table 32; Figure 25)

Previous workers have shown that the injection site should not influence the calculated cardiac output by the dye-dilution method. The present results confirm this, and further confirm the accuracy of the dye method in comparison with the Fick method.

Since this sixth recovery period differed from the other five recovery periods only in the site of dye injection, similarly good agreement between Fick and dye results was expected. The correlation was good ($r = 0.988$) and the regression coefficient ($b = 0.999$) did not differ significantly from unity ($1.0 > P > 0.90$). Inspection of the appearance times in this group, however, (Table 32), shows that the time available for stabilization of the spectral absorption of the indocyanine green-blood mixture when dye was injected into the pulmonary artery and sampled from the aortic root at times came dangerously close to the minimum of one to two seconds (Fox and Wood, 1960; Sinclair et al.,1960;

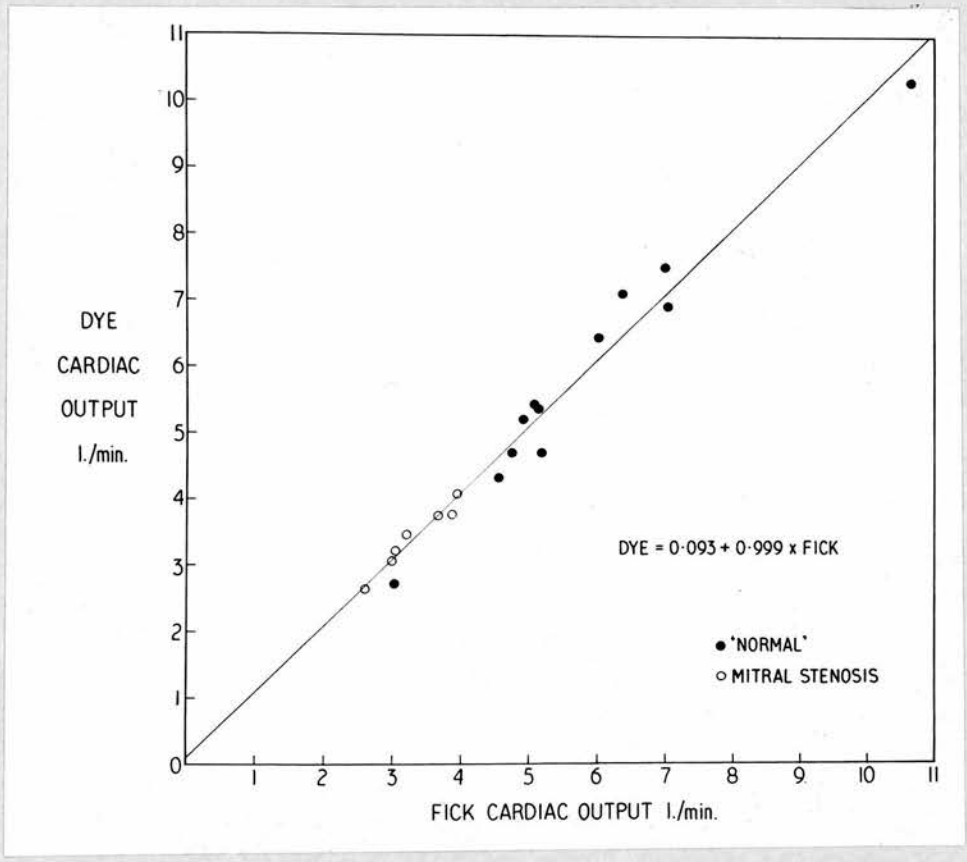

Figure 25: Comparison of simultaneous Fick and dye methods for cardiac output during sixth recovery (P.A. injection)

Bassingthwaighte et al., 1962). The times given in the table are corrected ones, however, and actual registration of dye-dilution curves took place 0.8 seconds later when the blood reached the centre of the densitometer cuvette, by which time spectral stabilization had apparently occurred.

The Justification for Claiming a more Basal State following Exercise

Donald et al. (1955) and Bayer, Richards, Metcalf and Gunther (195 have shown that measurements of resting cardiac output made after a short period of exercise are more truly basal than those made straight after positioning the catheters. Since the present study required absolute stability, this was best achieved when the subjects were in as basal a state as possible. The definitive dye-Fick comparison was therefore started twenty minutes after exercise.

A comparison of the dye-dilution results for cardiac output before and after the exercise period (i.e. initial rest period compared with first post-exercise recovery period) shows that, in almost all subjects studied, the cardiac output was lower following exercise, thus confirming the findings of the previous workers mentioned (Figure 26).

The Reproducibility of Consecutive Dye-Dilution Values for Cardiac Output in a Stable Subject

Numerous previous workers have performed serial cardiac output determinations by either the dye or the Fick method in apparently basal subjects in an endeavour to show how little the cardiac output of a resting subject alters. The accuracy of their interpretations has

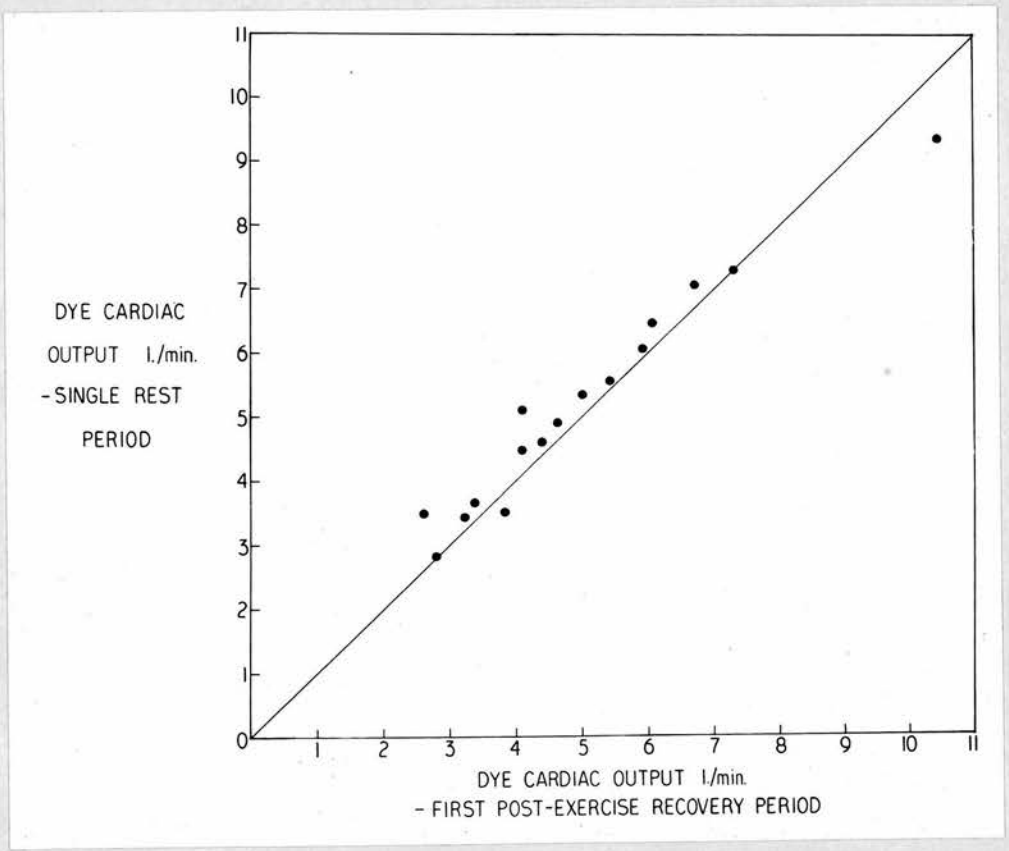

Figure 26: Comparison of resting cardiac outputs (dye method) before and after exercise

depended to a great extent on the accuracy with which they could measure cardiac output by their techniques. The present study is not intended to show how stable the cardiac output is in an apparently basal subject, because examination of the results (Tables 5 - 29) illustrate that subjects vary considerably in this respect. More relevant to this study is the accuracy with which consecutive cardiac outputs can be measured when the cardiac output is not changing. Regarding the heart as a mechanical pump preset to deliver an absolutely constant flow, how closely will consecutive dye-dilution values for flow agree while the pump output remains constant?

Examination of the results suggested that where the heart rate changed by less than two beats per minute between consecutive cardiac output determinations measured at one or two minute intervals, the cardiac output had probably not changed significantly between the two readings. All such pairs of consecutive cardiac outputs within each of the five recovery periods in all subjects were plotted in Figure 27. The 95 per cent. confidence limits were found to be 0.11 per cent. These results, like those of the dye-Fick comparison, confirm the accuracy of the dye-dilution method described.

General Criticisms of the Fick Method

Two assumptions are necessary for the validity of the Fick method:

1. The lungs neither extract oxygen from the blood nor eliminate carbon dioxide into the blood as it flows through the pulmonary circulation. This has been shown to be a valid

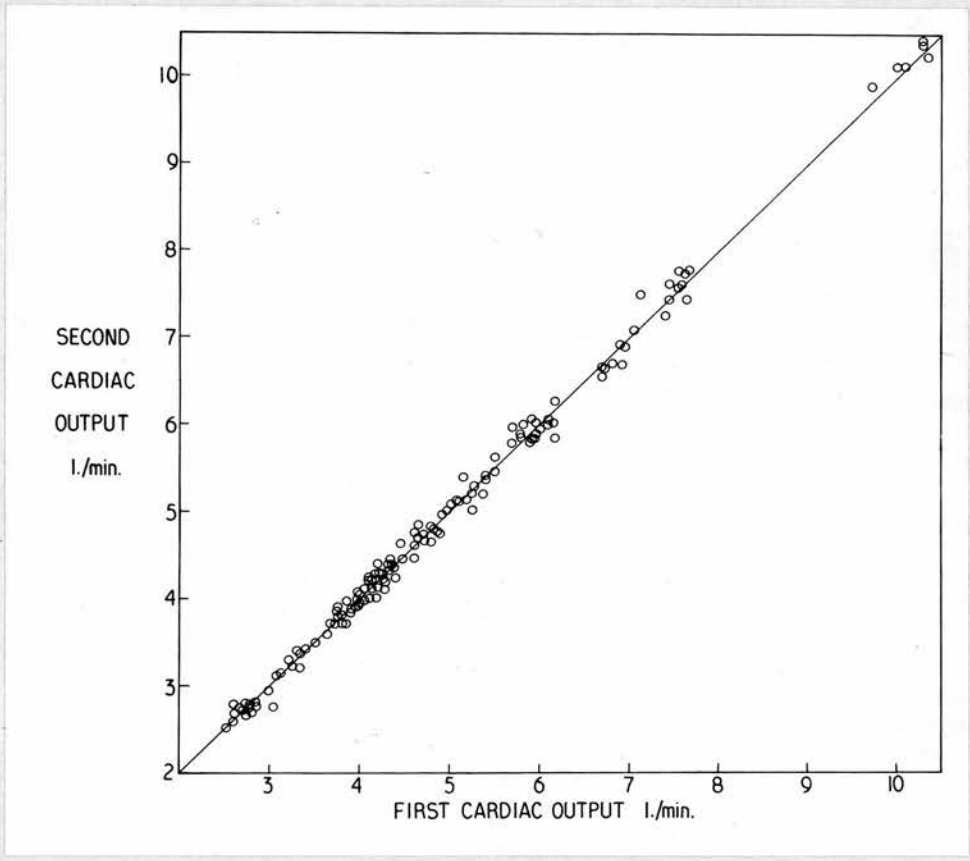

Figure 27: Pairs of consecutive cardiac outputs (dye method) in which heart rate differed by <2 beats per minute.

assumption by Mitchell and Cournand (1955) who demonstrated negligible lactic acid production by the lungs.

2. The mixed venous blood samples truly represent blood returning to the lungs. Pulmonary arterial samples have been shown to be adequately mixed (Cournand, Riley, Breed, Baldwin and Richards, 1945; Warren, Stead and Brannan, 1945; Sirota and Gordon, 1954).

The oxygen uptake actually measured during a Fick estimation is the volume of oxygen taken up per minute from the gas expired at the mouth. If this quantity does not equal the oxygen taken into the blood during the period of expired air collection, the method will be in error (Fritts and Cournand, 1958). The assumption is therefore necessary that the volume of nitrogen in the lungs remains constant during the expired air collection, which would not occur during an unsteady state such as occurs during the transition from breathing air to the breathing of gas mixtures other than air (Fishman, McClement, Himmelstein and Cournand, 1952; Nahas, Visscher and Haddy, 1952; Lousen and Demeester, 1953).

Hypoventilation or hyperventilation during the expired air collection would respectively decrease or increase the alveolar oxygen concentration. This would not necessarily represent the oxygen taken up by the blood although it would be measured as oxygen uptake at the mouth. Such errors are greatest when irregularity of respiration occurs at the beginning or end of an expired air collection, and are minimized by long periods of expired air collection.

A change in the respiratory level and hence the functional residual capacity of the lungs during the expired air collection may cause a false calculated oxygen uptake. Such a change will alter the supposedly constant volume of nitrogen in the lungs, and, if the functional residual capacity increases, a quantity of oxygen uptake will be measured at the mouth which has not entered the blood (Stow, 1954; Fritts and Cournand, 1958).

The A-V oxygen difference is intended to represent mean values over the period of expired air collection. The more samples taken the more truly does the value of the resultant pooled sample or average value of the individual samples approach the existing mean oxygen saturation during the time period. The least acceptable situation would be that in which only one arterial and one mixed venous sample were taken, and the best would be continuous withdrawal of arterial and mixed venous blood during the air collection to obtain a time average mean sample. As there are cyclic changes in the circulation rate dependent on the respiratory and cardiac cycle, constant rate sampling will not allow weighting of the blood samples taken when the flow is fast as compared with those taken when the flow is slow. Theoretically, a volume average mean would be preferable (Visscher and Johnson, 1953; Fritts and Cournand, 1958), as the volume of blood sampled should be proportional to the volume of blood passing the sampling catheter tip. When both cardiac output and A-V oxygen difference are changing the use of a time average sample will cause errors. These errors are relatively

unimportant during rest and occur only when large changes of cardiac output and A-V oxygen difference are taking place, as at the beginning of exercise (Visscher and Johnson, 1953; Stow, 1954; Wade and Bishop, 1962). They are similarly small during steady state exercise if measurements are made shortly after the second minute of exercise (Donald et al., 1955).

A further source of error in the determination of A-V oxygen difference is the known swing of oxygen saturation of mixed venous blood in relation to the respiratory cycle (Wood et al., 1955). This can best be overcome by sampling over several respiratory cycles.

In certain diseases such as bronchiectasis, congenital pulmonary stenosis and occlusion of a major pulmonary artery the bronchial blood flow is greatly increased. Bronchial arterial blood in considerable amounts enters the alveolar circulation and drains via the pulmonary veins. The Fick method cannot measure this flow and measures more nearly pulmonary arterial flow (Fritts and Cournand, 1958).

It has been argued that the procedure of cardiac catheterization necessary to obtain Fick estimates of cardiac output so upsets the subject that the cardiac output is not a truly basal one, but that of an apprehensive subject (Starr, 1945; Hauch and Danneel, 1954; Emmrich, Steim, Klepzig, Musshoff, Reindell and Baumgarten, 1958). The present dye-Fick comparison did not set out to support or refute this argument, but evidence has been presented showing that more basal conditions are obtainable following a short period of exercise.

Huckabee and Judson (1958), and Wade and Bishop (1962) showed that the presence of the catheters necessary for the measurement of cardiac output by the Fick method does not cause significant change of the basal metabolic rate or cardiac output respectively over long periods. The repeatability of the method further justifies the negligible effect of the procedure on the cardiac output.

Shortcomings of the Dye-Dilution Method used in this Study

General criticisms of the dye-dilution method have already been discussed in the introductory sections and during the discussion of the components of the present method. This section will therefore specifically examine imperfections in the system used in this study.

The Injection System: Two aspects of the injection system could be improved upon; one is the duration of the injection, and the other the timing. Although the injection time was short, ranging from 0.2 to 0.6 seconds in the present study, since it was manually operated, it was not identical each time. While this slight variation does not in any way affect the calculation of true cardiac output from the dye curves, provided that the duration of the injection is less than half the appearance time (Hoffman and Shillingford, 1957), it can affect the calculation of mean transit time and central blood volume as a result, if it is unduly long. Moreover, if the use of an empirical formula for calculating cardiac output were contemplated, its parameters would be based on such values as buildup time and peak concentration, which would be valid only if injection duration were constant.

In a subject with a fast, shallow respiratory rate and a tachycardia the timing of the injection with the cardiac and respiratory cycle is unimportant, but at slower cardiac and respiratory rates, however, it could well be important. Work by Brecher and Mixter (1953), and Brecher and Hubay (1954) suggests that considerable changes in pulmonary artery blood flow occur during the respiratory cycle. Opdyke and Sniffen (1959) quote a variation of cardiac output of as much as 30 per cent. during the respiratory cycle, and have themselves demonstrated a 20.8 per cent. difference in cardiac output when dye was injected during lung inflation, compared with when it was injected during the expiratory pause in artificially ventilated anaesthetized dogs. Hoffman, Guz, Spotts and Weirich (1960), using an electromagnetic flowmeter, claim a 50 per cent. variation in stroke volume with the phases of the respiratory cycle. In conscious human subjects the respiratory fluctuations are probably not nearly as great, but, with a slow respiratory rate two consecutive dye curves, with injections at different phases of the respiratory cycle, could give considerably different values for cardiac output. On the same principle, in a patient with an extreme bradycardia as in complete heart block, who is maintaining an adequate cardiac output by a greatly increased stroke volume, the timing of the injection with the cardiac cycle is of similar importance.

From the above considerations it is therefore more accurate, especially where comparisons of cardiac output during a changing state are to be drawn, that all injections should be made at precisely the same phase of the respiratory and cardiac cycles. With this in mind,

Grace et al. (1957), Warner and Toronto (1958), Opdyke and Sniffen (1959), Lindberg, Sutterer, Marshall, Hedley and Wood (1960) have devised solenoid-controlled, pneumatically-activated dye injection assemblies, which can deliver the injectate within a constant time, phased with respiration and triggered by the R wave of the electrocardiogram.

The Errors of Monchromatic Densitometry: The Waters XC-250A densitometer is a monochromatic densitometer, and the dye concentration is therefore measured against a background of blood, the optical density of which is itself capable of being changed by a variety of non-specific factors, all of which can interfere with the recorded dye concentration itself. As has already been mentioned, the Waters XC-300 will overcome these problems, and more will be said of this instrument in a later section. In the present study, however, the non-specific optical density changes which could interfere with the recording of dye concentration were appreciated, and the errors of monochromatic densitometry were therefore reduced. One factor, however, which could not be avoided was that with the fast dynamic response of the system used the curves were markedly pulsatile (Figure 1). The proper interpretation and analysis of pulsatile changes in blood density have not been completely explained (Heller et al., 1951), although they may be a manifestation of the effect of pressure or flow on the distribution of the erythrocytes. The phenomenon reduces the accuracy with which fixed points such as appearance time and peak concentration can be measured on the curve, but what is more important to the accuracy of the technique is what procedure should be adopted to assess true dye concentration (Dow, 1956). Should a

ballistic integration by a slow galvanometer, as employed in Wood's laboratory, or an arithmetical mean line drawn by inspection be used to give a true volume-average concentration? The latter method was used in the present study, but no theoretical proof can be offered of the validity of the procedure.

The Error of Failing to Plot to Infinity: As discussed under the theory of the method, failure to take concentration readings to infinity in calculating the area of dye curves may cause underestimation of curve area, and so systematic overestimation of flow. The error has been estimated by previous workers to be between + 3 per cent. and + 4 per cent. (Kinsman et al., 1929; Thorburn et al., 1959; Hoffman, 1960). It was explained earlier that there is no relationship between the systematic error and the magnitude of flow, because of the concomitant change in curve geometry with flow. In the present study calculations were not taken to infinity, but it is not possible to state the average concentration to which readings were taken for the following reason. A separate calibration figure in milligrams per litre was not used in the actual measurement of the dye curves of each subject, as a certain error will always arise in the construction of a measuring card marked in milligrams per litre for each subject. Instead, a set-square, ruled in millimetres, was used for each reading at fixed time intervals. These readings, taken to the nearest tenth of a millimetre, were used in the semilogarithmic replot, and eventually summed. The sum was then multiplied by a factor derived from each patient's individual calibration to convert centimetres into milligrams per litre. Calibrations did not,

however, vary much between different subjects, and generally one centimetre was equivalent to a concentration of approximately 0.5 mg./l. Readings were in fact taken to 1 mm. levels, which were therefore equivalent to approximately 0.05 mg./l. concentrations. As peak concentrations averaged approximately 6 mg./l., and readings were taken to a lowest concentration of approximately 0.05 mg./l., this represents approximately $^{1}/_{120}$th of peak concentration. Thorburn et al. (1959) estimated their error in calculating cardiac output to be + 4 per cent. by plotting to a concentration of $^{1}/_{40}$th of peak concentration in model experiments. Very approximately therefore, the error in the present cardiac outputs would be expected to be + 1 per cent. from this failure to plot to infinity.

The Observer Area in Calculating Curve Area: As was discussed under 'Methods', the sensitivity of the control unit could be increased or decreased over a preset range (Table 1). This meant that, in heavy exercise with high flows, an otherwise small curve could be enlarged to the same dimensions as resting curves. In addition, the paper speed could be varied to give the downslope an angle similar to the optimum chosen for resting curves, thus enabling readings to be taken on a convenient scale and a less vertical slope, all aiding accuracy of measurement. In curves of short duration, such as during exercise, readings were taken at half-second intervals to increase the accuracy of calculating area. Any error of mensuration should therefore be similar for all curves in any one subject, irrespective of the flow, but should be minimized by the adjustable paper speed and sensitivity control.

Errors are most likely to occur in the choice of the extrapolation, and reference to observer error was made in the section on the theory of the method of a limited study by Nylin and Hedlund (1958), in which three different observers calculated cardiac output from three different dilution curves to the nearest 0.1 l./min. Their calculated means were 6.0, 6.4 and 7.0 l./min., but they were constructing their curves from intermittent sample values for concentration. With the much-improved continuous recording technique used in the present study, errors of this magnitude would be most unlikely. A recent, more comprehensive study by Sleeper et al. (1962) has investigated the error of mensuration more fully. Using indocyanine green and Gilford densitometers, they estimated the cardiac output over a range of flows varying between 4 - 15 l./min. By feeding two densitometers from the same catheter via a Y-connection and calculating the cardiac output from 90 simultaneous pairs of curves, and comparing simultaneous paired curves obtained from a densitometer feeding two amplifiers in 38 estimations, they were able to separate out the components of the error due to instrumentation plus mensuration from those due to mensuration alone. They found the latter error to be 6 per cent. of cardiac output, and that it was largest when the cardiac output was high and the curves therefore small; this was not a factor in the present system where sensitivity and paperspeed adjustment maintained the curve geometry relatively constant irrespective of cardiac output.

While no study of this type has been attempted in the present system, a limited investigation has been conducted. Since the author measured all the dye curves used in this study, it was necessary to demonstrate his error of measuring such a curve, and also whether his measured area differed systematically from the values obtained by other observers. The typical dye curve illustrated earlier in Figure 1 was photostatted to obtain 30 identical copies. The author measured the area of ten of these curves as did two colleagues well-acquainted with the method. Photostats of the same curve eliminated any discrepancy in curve size which would result from using curves simultaneously inscribed from more than one densitometer or recorder, which were not perfectly balanced. The use of only one curve was felt justifiable because paperspeed and sensitivity adjustments avoided discrepancy in overall curve area and slope between different subjects, which meant that a single curve was representative of almost all curves produced in this study. Each observer chose a suitable baseline, smoothed the pulsatile record freehand, measured the curve at half-second intervals, replotted semilogarithmically and chose a suitable extrapolation before arriving at the curve area. All stages of curve mensuration were therefore included. The observed curve areas in cm. sec. are presented in Table 34:

TABLE 34

	Author	Observer A	Observer B
Curve areas in cm./sec.	97.12	99.68	98.70
	99.64	99.80	98.95
	97.22	100.23	99.00
	96.63	100.54	99.35
	99.28	100.26	98.80
	98.77	100.22	98.70
	97.14	101.16	99.10
	99.99	101.39	99.25
	98.70	101.59	98.70
	97.63	101.32	99.05
Mean value	98.21	100.62	98.96
S.E. of single observation	0.38	0.22	0.07
S.D.	1.20	0.69	0.23

DISCUSSION OF THE VERSATILITY OF THE DYE-DILUTION METHOD AS DESCRIBED

The Applicability of the Method of Estimating Cardiac Output

Evidence has been presented for the accuracy of the dye-dilution technique described. The data presented have been obtained with central injection and sampling sites, but, as has already been discussed, there is no reason to believe that slightly more peripheral injection would reduce the accuracy of the method. To obtain the greatest possible versatility, radiographic screening of the catheters is undesirable. Positioning of the arterial catheter tip in the aortic root without radiographic screening has already been discussed. "Blind" insertion of a similar catheter of suitable length into the median basilic vein by the same technique might be expected to attain a fairly central position without the need for radiological control. This technique has frequently been practised in a current study in this department on patients in the ward, where radiographic screening facilities are not available. Pressure traces from the catheter tip have commonly shown a right atrial pattern, confirming the belief that a fairly central injection site, certainly somewhere within the thoracic venous system, can be achieved by such a blind procedure. The insertion of one arterial and one venous nylon catheter each of 1.34 mm. external diameter by a Seldinger technique removed the need for cut-down procedures and enables cardiac output measurements to be obtained with precision in situations where radiographic screening facilities are not available. Dow (1956) has listed the wide range of situations in which cardiac output has been measured by the dye-dilution method. These examples illustrated that

the dye-dilution method is equally applicable in all the situations in which the Fick method can be employed, but that there are several important instances in which the Fick is unsuitable, whereas the dye-dilution method remains suitable. The procedure is so safe and simple that it is applicable to patients in whom a Fick procedure would be unjustifiably complicated, hazardous or unreliable, and in situations where such a procedure would not be mechanically practicable. An excellent example of the latter is given in the study by Lindberg et al. (1960) who were able to measure cardiac output in subjects during headward acceleration in the human centrifuge by such a dye-dilution technique. Its suitability in sick patients has been proved in this department by recent studies on patients shocked by myocardial infarction or traumatic injury, in whom a Fick procedure would be undesirable, yet in whom the dye-dilution technique causes little disturbance, and is perfectly safe. Toscano-Barboza, Kirklin, Swan and Wood (1957) have demonstrated the use of the technique during surgery, and Merriman et al. (1958), Johnson (1951), Lee et al. (1953), and Etsten and Li (1954) have applied it during anaesthesia. Prec and Cassels (1955) have employed the technique in newborn infants to assess the time of closure of the ductus arteriosus and the foramen ovale, although they were obliged to use an earpiece because of the impracticability of arterial sampling in the newborn.

The dye-dilution method removes the need for a mouthpiece to collect the expired air as is necessary in the Fick method. This allows studies in patients in whom a mouthpiece would not be tolerated, due to respiratory distress or unconsciousness. Stability is also improved by avoiding a

mouthpiece, as many patients tend to ventilate unnaturally through a mouthpiece, and so enter an unsteady state which is undesirable during a Fick estimation of cardiac output. The presence of an unsteady state invalidates the Fick method (Visscher and Johnson, 1953; Nahas et al., 1953), as has been discussed in the section dealing with the errors of the Fick method. The dye-dilution method, however, remains perfectly valid under such circumstances.

Rapidly changing states of cardiac output, such as during non-steady state exercise, cannot be measured by the Fick method with any great accuracy, while the dye-dilution method remains valid, as will be illustrated in a later section. Shortlived cardiac output responses to drugs likewise cannot be assessed by the Fick method. Very rapid changes in cardiac output, such as during a Valsalva manoeuvre, are not even measurable by instantaneous injection dye-dilution techniques. Continuous infusion techniques can, however, overcome this problem by registering second-by-second fluctuations in flow (Crowley, Grace, Fox and Wood, 1956; Swan, Crowley and Wood, 1956; Birkhead, Marshall, Swan and Wood, 1957). As was discussed earlier, recirculating indicator can be "backed off" by sampling via a second densitometer, but the necessary time corrections become erroneous during a changing state. This could be overcome by an independent series of instantaneous injection curves during exactly the same manoeuvre to establish the time corrections which could then be applied to the definitive experiment in which flow was measured by constant infusion. The instantaneous injection technique described in this study could readily

be converted for constant infusion use.

Discussion of the short period over which cardiac output can be measured by the instantaneous injection technique, and the even shorter period possible with the constant infusion technique, focuses attention on the undesirability of single cardiac output estimations by the dye-dilution method being used as representing the true cardiac output during a steady state. Numerous studies have been quoted in an earlier section where single dye dilution cardiac output values have been compared with cardiac outputs obtained by the Fick method, obtained over several minutes. It was pointed out that such comparisons were erroneous by virtue of the widely different time periods over which cardiac output was measured by the two methods. Figure 28, obtained from results during recovery in subject R.A., illustrate the considerable swing of resting cardiac output over five four-minute periods during an apparently steady state. Individual dye-dilution values for cardiac output would not have been truly representative of the cardiac output during each four minute period, yet the average of the five values approximates the true average much more closely. Numerous investigators have failed to appreciate this fact. They have deduced that the value for cardiac output obtained from one or two dye-dilution curves can safely be taken to represent the control cardiac output, which they have then compared with values obtained during a state altered by administration of drugs, exercise or some similar manoeuvre. This may be true only where the heart rate can be shown to be absolutely steady, as was demonstrated in a previous section (figure 27). A basal

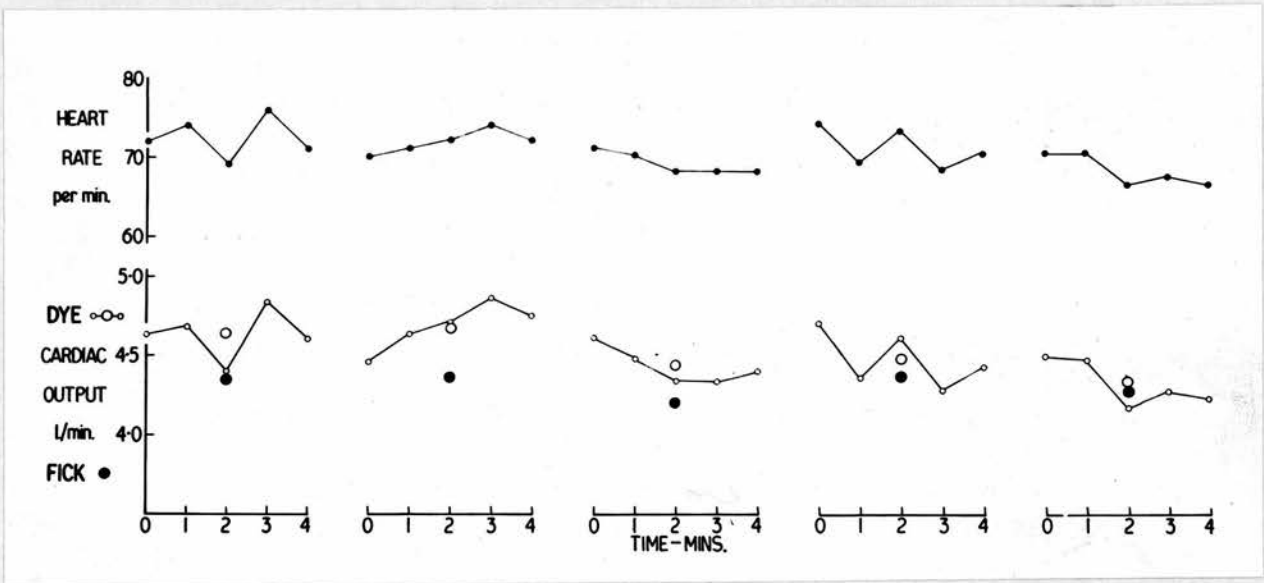

Figure 28: Subject R.A.: Minutely cardiac outputs and pulse rates during recovery

cardiac output obtained by a four-minute Fick procedure would be more appropriate than a single dye-dilution value under such circumstances. Sufficient consecutive dye curves would be equally adequate however, and the dye-dilution method would be necessary to follow any rapid change in cardiac output during the subsequent changing state.

The Rôle of Dye-Dilution Techniques in the Diagnosis of Congenital Cardiac Defects

Shortly after the first, nearly simultaneous, descriptions of methods for the continuous recording of Evans blue dye concentration from Johns Hopkins University (Friedlich et al., 1950) and the Mayo Clinic (Nicholson et al., 1950; Knutson et al., 1950) came the first description of the typical contours of normal dye-dilution curves compared with those in intracardiac shunts (Nicholson et al., 1951). Since that time it has been largely due to the work of Wood and his colleagues that the use of indicator-dilution techniques has been established as a most important tool in the diagnosis of congenital heart disease.

Recordings of arterial indicator-dilution curves obtained after injections of indicator at selected sites on the right side of the heart provide one of the most sensitive practical methods currently available for the detection, localisation and quantitation of right-to-left shunts (Nicholson et al., 1951; Swan and Wood, 1953; Swan, Zapata-Diaz and Wood, 1953; Swan, 1954; Swan et al., 1954; Silver et al., 1956; Wood et al., 1957; Fox et al., 1957; Fox and Wood, 1960 b).

Measurement of relatively small right-to-left shunts, some of which

cannot be detected by the slight decrease in the oxygen saturation of arterial blood, is possible. It is possible to detect and obtain some idea of the magnitude of shunts of less than five per cent. of the systemic blood flow. In the presence of balanced pressures between the cardiac chambers, or of extremely small defects, the detection by this method may frequently be the only evidence obtained at cardiac catheterisation of the presence and location of the defect in question (Swan et al., 1953).

Arterial dilution curves may not detect left-to-right shunts of less than twenty per cent. of pulmonary blood flow by alteration of their contour (Broadbent and Wood, 1954; Carter, Bajec, Yannicelli and Wood, 1960), and hence venous dilution curves are more appropriate. Nevertheless, they do have some quantitative value (Broadbent and Wood, 1954; Ramirez de Arellano, Hetzel and Wood, 1956; Carter et al., 1960) as the degree of shunt can be roughly obtained by means of the forward-triangle method (Ramirez de Arellano et al., 1956); Dow's formula (Dow, 1955); the disappearance ratios of indicator concentration $\frac{C(p + BT)}{Cp}$ and $\frac{C(p + 2BT)}{Cp}$ (Carter et al., 1960), or measurements of the ratio of least concentration to systemic recirculation concentration of the curve (Wood, 1962 a).

Venous dilution curves recorded from selected sites on the right side of the heart by a double-lumen or two-catheter technique, provide the most sensitive practical method currently available for the detection, localization and quantitation of left-to-right shunts (Broadbent and Wood, 1954; Swan, Hetzel, Burchell and Wood, 1956; Fox and Wood, 1957 b; Wood et al., 1957; Hyman, De Graff and Quiroz, 1959).

Diagnosis and measurement of blood flow and shunts by means of venous dilution curves attain the highest degree of accuracy when an arterial dilution curve is recorded simultaneously. Such curves can be used for determination of systemic and pulmonary blood flows and the magnitude of the left-to-right shunts (Swan, Fox, David and Wood, 1957; Russell, Donald, Moersch and Marshall, 1958; Russell, David, Donald, Wood, 1958; Wood, Swan and Marshall, 1958).

Certain distinct advantages of indicator-dilution techniques should be emphasised. The measurements required are recorded simultaneously over a period of less than a minute, and provide an accurate comparison of the haemodynamics of the pulmonary and systemic circulations, information which may be of considerable practical value in selecting patients with intracardiac defects for possible surgical repair. The method does not require blood-gas analysis or active co-operation by the patient. With the use of indocyanine green as an indicator (Burchell, 1960) the method is not interfered with by the presence of foreign gases or variable oxygen saturation in the blood, so that the techniques can be applied readily during anaesthesia (Toscano-Barboza et al., 1957), and during clinical surgical measures. No accessory determinations of respiratory gas exchange are required. In addition to the use of venous indicator-dilution techniques for the detection and measurement of relatively simple cardiac defects, these techniques are also applicable to the elucidation of multiple or complicated congenital or acquired cardiac defects.

The techniques have particular application in congenital or acquired

lesions affecting the left side of the heart, not ordinarily accessible to study by conventional right heart catheterization. By means of techniques that have been developed in recent years, it is now possible to gain access to any chamber of the heart or the great vessels for either the injection of indicator or the withdrawal of the resulting dye-blood mixture (Woodward, Swan and Wood, 1957; Morrow, Braunwald and Ross, 1960; Ross, Braunwald, and Morrow, 1960; Sinclair, Newcombe, Donald and Wood, 1960). When the need arises in cases of complicated or multiple cardiac defects involving the left side of the heart, it is possible to utilize these techniques in conjunction with one another with an acceptable degree of safety. This allows study of any of the four heart valves or cardiac chambers during one procedure. This type of complicated investigation need be carried out in only a small number of specially selected patients with multiple lesions of the left side of the heart and perhaps also with involvement of the right side of the heart, in whom the information required to establish a diagnosis and determine the feasibility of surgical repair cannot be obtained with certainty by simpler techniques (Swan, Burchell, Linder, Birkhead and Wood, 1958). With reference to the possible dangers of the dye methods in the diagnosis of cardiac lesions, Wood reports that in more than 3,000 such investigations in his laboratory there have been no deaths, and little or no morbidity (Wood, 1962 a).

Although indicator-dilution techniques are of considerable diagnostic value as an independent method, they attain their greatest value when used in conjunction with cardiac catheterization. The latter embraces pressure

and oxygen saturation recording in selected sites in the cardiac chambers and great vessels, as well as selective angiocardiography (Amorim, Weidman and Wood, 1960). Angiocardiography is in reality an indicator-dilution technique, the contrast medium being the indicator, and the X-ray apparatus the detector. The difference from usual indicator-dilution techniques is that the output of this detector is usually a subjectively appraised visual image of a segment of the circulation, rather than a quantitative record of the concentration of the indicator at a specific localized site in the circulation. Where indicated, certain other ancillary techniques should be available in the diagnostic armamentarium, such as intracardiac phonocardiography (Soulie, Bouchard and Laurens, 1959; Lewis, Moghadam, Deitz, Wallace, Brown and Khalil, 1960), and intracardiac electrocardiography (Hernandez, Rochkind and Cooper, 1958). Each of the above is indispensable for the performance of the best possible type of diagnostic cardiac catheterization, and none of the techniques should be used to the exclusion of the others.

In recording of dilution curves from the cardiac chambers and great vessels, where stepwise changes in indicator concentration occur with each heartbeat, the contours of the curves are badly damped versions of the actual variations in indicator concentration occurring at the tip of the cardiac catheter. Withdrawal rates have obviously to be reduced to prevent cavitation when sampling through the necessarily longer catheters used for entering the right heart. This obviously increases the volume-flow ratio, and reduces the dynamic response of the sampling-detecting-recording system

described in this thesis. Fortunately, the practically important diagnostic applications of venous dilution curves for detection of intracardiac shunts are based on dimensions which can be measured with acceptable accuracy, despite the unavoidable sacrifice of optimum fidelity of inscription. It is nevertheless desirable to strive for the best possible system, as is admirably illustrated by an example in a paper by Wood and co-workers (Wood et al., 1957) where, turning to the arterial system, ear oximetry gave a dilution curve so damped as to be uninterpretable, whereas the cuvette oximeter, with its far better dynamic response, detected the abnormal pattern of a persistent common atrioventricular canal in a ten-month old child. It is true to say that particularly in children with abnormal circulatory pathways, systems such as ear oximeters may not reproduce with accuracy the sudden brief changes in concentration of dye associated with their rapid circulations.

Dye-Dilution Curves in Valvular Incompetence

Kopelman and Lee (1951) first drew attention to the dispersion effect of valvular incompetence on dye-dilution curves. As pointed out by Korner (1961), this is not specific to valvular incompetence, but occurs in central left-to-right shunts as well, and valvular incompetence can in fact be regarded as a very limited type of left-to-right shunt. Despite the distortion effect of valvular incompetence, the curves have a well-defined downslope, and valid estimates of cardiac output and central blood volume are obtainable from the curves (Korner and Shillingford, 1955; Korner, Thorburn and Edwards, 1959). With incompetent semilunar valves, the volume

ejected during systole includes the forward as well as the regurgitant stroke volume, the latter returning to the ventricle during diastole. When the atrio-ventricular valves are incompetent, part of the systolic stroke volume is ejected forward by way of the semilunar valves, and part of it regurgitates into the atrium via the incompetent tricuspid or mitral valves (Muller and Shillingford, 1955). When dye is injected at, or upstream to the zone of incompetence, its rate of movement is thus increased in one phase of the cardiac cycle, and retarded in another, the phase depending on whether the semilunar or atrioventricular valves are involved; hence the increased dispersion of dye (Korner and Shillingford, 1955; Conn et al., 1957; Woodward, Burchell and Wood, 1957; Korner et al., 1959). This dispersion manifests itself by an earlier appearance time, a lower peak concentration, a shorter peak concentration time, and a more prolonged downslope.

Using the above observations, the site of the incompetent valve can be localised by the use of multiple sampling or injection sites (Korner and Shillingford, 1955; Braunwald, Tanenbaum and Morrow, 1957; Wright and Wood, 1957), as curves obtained with injection of dye beyond a competent valve downstream to the incompetent valve have a normal contour, whereas curves are abnormal if injection is made at the incompetent valve, or upstream to it. Similarly, with the multiple-sampling technique indicator is injected on the venous side of the circulation, and curves recorded upstream to the next proximal competent valve are normal, whereas abnormal curves are obtained when sampling at, or downstream to, the incompetent valve.

Valvular stenosis alone does not alter the dye curve contour, but may, if severe, cause the curves to be symmetrically enlarged, because of a low cardiac output. One of the most familiar problems facing cardiologists however, is the degree of incompetence present in association with known valvular stenosis, because of the importance of accurate assessment pre-operatively. Several qualitative tests have been described to assess the degree of valvular incompetence present. Some of these express the degree of distortion of the time components, downslope and recirculation peak of the dye curve; others are based on the assumption that mixing is closely related to regurgitation and they attempt to isolate the effect of increased mixing from the results of increased cardiac volume and reduced cardiac output (Nixon and Snow, 1962).

The former group includes several methods of expressing the flattened downslope of dye curves where valvular regurgitation is present. They include the ten-second disappearance ratio (Levinson, Carleton and Abelmann, 1959), and the time for a tenfold decrease in dye concentration (Hancock, 1959). Also in this group is the method of Warner (1962) in which the slope is plotted against the buildup time. Wood and Woodward (1957) measured the ratio of the least concentration before the recirculation peak (C_L) divided by the height of the recirculation peak (C_R), a method which has yielded the best results of all the indices devised (Woodward et al., 1957; Marshall, Woodward and Wood, 1958; Nixon and Snow, 1962).

In the second broad group of formulae which try to isolate the effects of increased mixing from the effects of increased heart volume and decreased

cardiac output are the following: the ratio of the disappearance time to the buildup time (Woodward et al., 1957); the ratio of the disappearance time to the mean transit time (Levinson et al., 1959); the disappearance ratio (the dye concentration measured on the downslope at an instant in time found by adding the buildup time to the instant of peak concentration, divided by the peak concentration) described by Fox and Wood (1957); and finally the ratio of spread to appearance time (Shillingford, 1958). In this latter ratio the spread is represented by the width of the semi-logarithmic replot of the curve in seconds at a point of one-tenth peak concentration. Resnekov (1962) has subsequently shown that the incomplete left atrial mixing which may occur in mitral regurgitation limits the efficacy of this simple index.

Warner (1962) suggested that some indices used to separate patients with mitral regurgitation are dependent on the relationship of the amount of smearing of the dye curve as it passes through the pulmonary circulation and left heart, to the smearing which occurs as the dye passes around the whole circulation or through the right heart and the systemic veins. The $\frac{C_L}{C_R}$ ratio of Wood and Woodward (1957) is just such an example. C_L depends only on events taking place as the indicator travels from the pulmonary artery to the systemic sampling site, whereas C_R is influenced by events occurring in the entire circulation. Warner believes that the success of this index depends on the selective dilatation of the left ventricle, left atrium, and possibly pulmonary vascular bed, as compared with the systemic

veins and right heart in patients with mitral regurgitation. He also suggested that his own method of plotting slope against buildup time depends on left heart dilatation out of proportion to changes in the pulmonary circulation which smear the dye curve by the time it enters the left atrium. Whatever the mechanism producing the changes in the various curve parameters in mitral regurgitation, none of the methods described consistently distinguishes the clinically difficult cases.

Although these qualitative methods are valuable in the pre-operative assessment of patients, quantitative data would be more valuable. Four different principles of quantitating regurgitant flow have been devised:

(1) The first of these was introduced by Korner and Shillingford in 1955, and although it has evoked considerable criticism, it awakened interest in a potentially most valuable contribution of indicator dilution techniques. Their method, known as the dispersion method, involves comparison of indicator dispersion in a system with valvular incompetence with that in an identical system with competent valves at constant flow and central volume (Korner and Shillingford, 1955, 1956; Woodward et al., 1957; Novack and Schlant, 1958; Korner et al., 1959, 1960).

In order to specify dispersion of indicator, Korner and Shillingford (1955) initially used the reciprocal of the downslope of the curve ($^1/_s$) as a convenient parameter: $^1/_s = \frac{t_2 - t_1}{\log c_1 - \log c_2}$, where t_1c_1 and t_2c_2 represent time and concentration co-ordinates of two points on the straight line downslope when the curve has been replotted semilogarithmically. If c_1 and c_2 are chosen one log cycle apart, and base 10 is used throughout

instead of $\log_e C$ in defining $^1/_s$ (Novack and Schlant, 1958), the denominator reduces to one, and $^1/_s$ becomes simply the number of seconds for the concentration to drop to one tenth of any given value on the straight line downslope. Since the slope of the downslope is affected by flow, central volume and valvular regurgitation, multiple regression equations were calculated from dye curves from normal subjects over a range of expected flows and central volumes. In any individual patient, therefore, the calculated cardiac output and central volume could be substituted in the regression equation to calculate predicted slope for that flow and central volume, and with the observed slope a measure of the regurgitant flow could be obtained. Total ventricular output (FF) was then obtained from the formula: $FF = CO \times \frac{1/s \text{ observed}}{1/s \text{ predicted}}$, and backflow (B) could be calculated from the relationship: $B = FF - CO$.

Because the accuracy of the regression equations probably diminishes in the low flow, high central volume range due to the paucity of such points in the data used in the equations, Korner and Shillingford (1956) suggested using the variance of the curve instead of the slope. The variance of the curve was obtained by the second moment of the frequency function. The curve was extrapolated to an arbitrary concentration, and the variance calculated as follows:

$$Vx = \frac{1}{\sum(c)} \left[\sum(ct^2) - \frac{\sum^2 (ct)}{\sum (c)}\right],$$

where Vx represents the variance of the curve (sec.^{2}), c the indicator concentration, t the time (sec.) after appearance time, and $\sum$ the summation of the bracketed term.

The variance of a curve obtained in valvular incompetence has a component ascribable to normal forward flow and central volume, and a component due to backflow: Vx observed = Vx (F;V) + Vx (B;V), where Vx observed is the calculated variance of the curve obtained during valvular incompetence, Vx (F;V) is the expected variance for the same forward flow and volume with the valves competent using the same injection and sampling sites, and Vx (B;V) is the component of variance due to backflow. The amount of backflow is given by the relationship:

$\frac{\text{Vx observed}}{\text{Vx (F;V)}} = \frac{\text{total flow}}{\text{forward flow}}$, where total flow is the sum of forward and backward flow. Forward flow and Vx observed can be calculated from the curves recorded during valvular incompetence by the usual methods. The expected variance, Vx (F;V), must be calculated from multiple regression equations relating the variance of the curve to forward flow and central volume, calculated for an identical system.

The following assumptions are necessary in the foregoing equations:

(a) The component of variance due to backflow adds to the component of variance for the same forward flow and central volume.

(b) The experimental conditions must ensure that the mean circulation time remains unaltered by the valvular incompetence so that the increased dispersion must be independent of the volume between injection and sampling sites (Korner et al., 1960). Experimentally, this requires that all dye particles must have the same chance of being either accelerated or retarded en route through the incompetent valve (Korner et al., 1959), as will be

achieved by injection upstream to the incompetent valve (Korner et al., 1960).

(c) The component of variance due to backflow must be directly proportional to the true backflow through the incompetent valve. To satisfy this assumption the dye must be injected close behind the zone of incompetence, so that the dye enters this region as a compact, undispersed mass. Dye injection far upstream from the zone of incompetence fails to satisfy this assumption. It was this error which led Hoffman and Rowe (1959) to the conclusion that the dispersion method was unreliable. When the injection site is in its correct situation there is a far greater difference in dispersion between a curve obtained with an incompetent valve and a normal valve, than when the volume between injection site and zone of incompetence is large, because in the latter case the dye is already widely dispersed before its arrival at the dispersing zone of incompetence. Korner et al. (1959) have shown that the degree of underestimation of the backflow using the incorrect injection site is related to the amount of initial dispersion of the dye between injection site and the incompetent valve, and that backflow can be estimated by taking this into account: $\frac{Vx \text{ observed} + d}{Vx\ (F;V)} = \frac{\text{total flow}}{\text{forward flow}}$, where d is the variance due to dispersion between injection site and zone of incompetence.

The dispersion is invalid for similar reasons where incompetence of more than one valve is present, and causes underestimation of backflow because the first incompetent valve disperses the dye, as did the volume between injection site and the zone of incompetence, so that the additional dispersion effect of the second incompetent valve is minimised.

In their model studies (Korner and Shillingford, 1956) showed that variance could be calculated from multiple regression equations relating the variance of the curve to forward flow and volume. In dogs, in which valvular incompetence was surgically produced, expected variance could be obtained from pre-operative studies with competent valves, and its calculation from "specific" regression equations gave reasonable estimates of backflow. In the clinical application of the method, however, such equations are not available for any given patient, and the value of Vx (F;V) must be arrived at by some other means.

Circulatory dimensions, which influence curve variance, vary between different subjects, and account for the poor results of calculating Vx (F;V) from "general" regression equations (Novack and Schlant, 1958; Woodward et al., 1957; Shillingford and Zoob, 1957). Just how unreliable such equations can be was shown by Warner (1962). In model experiments he showed the considerable effect on the time constant of the downslope of increasing the end-systolic residual volume of the ventricle from 50 - 100 ml. The effect of the regurgitant stroke volume was indistinguishable from the effect of increasing the end-systolic residual left

ventricular volume by an identical amount. No independent measurement of the effect of left atrial and left ventricular volumes can be made, and "general" regression equations would not take these into account. Quantitation of the volume of regurgitant flow in an individual patient is therefore subject to gross error due to these uncontrolled effects of variations in the volume and distensibility in the chambers upstream and downstream to the incompetent valve (Woodward et al., 1957; Marshall et al., 1958; Shillingford, 1959). The importance of these factors in determining the contour of a dye curve associated with a given degree of valvular regurgitation has been demonstrated in experiments using a circulation model by Hoffman and Rowe (1959). Although their injections were not made as near to the incompetent valve as would be desirable to reduce to a minimum any dispersion of the dye bolus prior to arrival at the dispersing incompetent valve, their studies showed the very definite influence on curve slope and variance of such factors as the size, shape and elasticity of the atria and ventricles, and the force, shape and direction of the regurgitant jet, for which allowance could not be made in Korner and Shillingford's method. They observed that the effect on dye dispersion was due not only to the amount of backflow, but also to the dilution of the regurgitant dye. The extent of this dilution depends partially on the factors mentioned above. If the regurgitant dye mixes poorly with the blood in the proximal chamber, as would be the case with a rigid atrium, it will be little diluted, and will rapidly be washed out with little resultant curve distortion. If, on the other hand, it mixes

well with a large volume of blood in an elastic proximal chamber, it will be greatly diluted, its washout prolonged, and the curve will show considerable dispersion. Phinney, Cotton and Shillingford (1960) have shown that the mixing of dye in the chamber proximal to the incompetent valve is increased by the degree of incompetence, and this effect is more apparent in larger atria, where mixing is otherwise incomplete. Since the residual volume of the left ventricle in aortic incompetence is probably less than that in the right atrium for an equal amount of tricuspid incompetence, the dilution of regurgitant dye may be less with an aortic lesion and so produce less alteration of the curve variance or slope.

It becomes quite obvious therefore that all the numerous factors which can influence dye dispersion cannot possibly be accounted for adequately by the method of Korner and Shillingford (1955, 1956), and that the degree of dye dispersion, where allowance for flow and central volume is made, is not related to the degree of valvular incompetence alone.

Korner et al. (1960) derived estimates of expected variance from the interrelationship between specific intercept values from different injection sites. They obtained normal curves by injecting downstream from the zone of incompetence, and so derived specific intercepts upstream from this zone.

An alternative method may be applied by the use of the ratio of the slopes of the probit-regression lines of the probit transformation of the curve. The dye curve, extrapolated to an arbitrary low concentration,

is integrated to obtain a cumulative frequency function, usually termed a distribution function (Kendall and Stuart, 1958). This distribution function has a sigmoid shape, but is not a Gaussian distribution function, as may be demonstrated by use of the probit transformation (Finney, 1947), which would transform a Gaussian distribution into a straight line. Two straight lines can, however, be fitted to this function, and the dye curve can therefore be considered as consisting of two overlapping, approximately Gaussian, components. Two probit-regression lines are fitted to these two components. The ratio of these probit-regression lines is determined, and provides a measure of the asymmetry of the curve (Korner, 1961). This ratio remains approximately constant in the presence of valvular incompetence (Korner et al., 1960), so that information about the dimension of the system can be obtained from the abnormal curves themselves. The expected variance for a given forward flow, volume, and probit slope ratio can then be calculated from a triple regression equation. This method is a great improvement on that using "general" regression equations, but not as good as the results using "specific" regression equations which, of course, is not practicable in patient studies.

The dispersion method has two potential sources of error. The first, the result of variation in the partitioning of the dye with the phasic nature of cardiac output, can be overcome by averaging estimates obtained from several successive curves. The second, the difficulty of estimation of Vx (F;V) has been partially overcome, and more elaborate statistical specification of the abnormal curves themselves may be possible

by predicting the regression function by calculation of the third-or-fourth-order moment of the frequency function (Korner, 1961).

(2) The second means of assessing valvular incompetence quantitatively is the method of multiple sampling (Milnor, 1957; Woodward et al., 1957; Conn et al., 1957; Marshall et al., 1958; Heiman, Blakemore, Conn, Jumbala and Woske, 1958; Bajec, Birkhead, Carter and Wood, 1958; Lacy, Goodson, Wheeler and Newman, 1959; Armelin, Michaels, Marshall, Donald, and Wood, 1960; Sinclair et al., 1960). In a case of mitral incompetence, dye is usually injected into the left ventricle, and dye curves are inscribed simultaneously from the left atrium, and aortic root or a peripheral artery. Lacy and McClure and their associates (Lacy et al., 1959; McClure, Lacy, Latimer and Newman, 1959), in their model studies used the formula: $\frac{\bar{Q}_B}{\bar{Q}_F} = \frac{\sum \bar{c}_{ad}}{\sum \bar{c}_{vs} - \sum \bar{c}_{ad}}$, where $\bar{Q}_F$ and $\bar{Q}_B$ represent forward and regurgitant flows respectively, during one cardiac cycle, and $\sum \bar{c}_{ad}$ and $\sum \bar{c}_{vs}$ represent the average concentration of indicator in the atrium during each ventricular diastole, and in the ventricle during each systole respectively.

Each of these concentrations is summed for the number of cardiac cycles necessary to clear the indicator from atrium and ventricle. Since diastolic atrial concentrations must be measured, undistorted curves are ideally necessary. Sampling from the left atrium in patients, however, requires a long catheter, which prevents optimum hydraulic characteristics, and a very damped record of actual systolic and diastolic variations in

concentration is obtained. Lacy et al. (1959) claimed good agreement between estimated backflows and observed backflows in their circulation model. Warner (1962) points out, however, that the equation is not true in the general case, because neither atrial indicator concentration nor forward flow from atrium to ventricle is constant in diastole, a necessary condition for the validity of the formula.

Sinclair et al. (1960) used the approach of measuring the ratio of the area under a dye curve obtained from the femoral artery to the area of a curve from the left atrium following left ventricular injection. They calculated regurgitant flow from the formula:

Regurgitant flow $= \frac{Q \times R.F.}{1 - R.F.}$, where Q represents the cardiac output calculated from the dye curve by the conventional Hamilton method, and R.F. represents the regurgitant fraction, which is the ratio of backward flow to the sum of backward plus forward flow, given by the ratio of the areas of the first parts of the curves recorded from the left atrium, and a systemic artery.

They found that the ratio of the total area under the two curves underestimated by 5.4 per cent. the regurgitant flow estimated by the hydraulic formula of Gorlin and Dexter (1952), using the pressure gradient measured during the experiment, and the cross-sectional area of the defect measured at post-mortem.

Several assumptions are necessary for the validity of the multiple sampling method:

(a) The concentration of indicator in the aortic root or the peripheral artery must represent the concentration in the ventricle.

(b) At the onset of ventricular ejection, the indicator must have been mixed in the ventricle with sufficient uniformity to ensure that its concentration in the fluid volume ejected forward is identical with its concentration in the regurgitant volume.

(c) Mixing in the atrium must be uniform by the end of diastole.

Woodward et al. (1957) showed that all the above assumptions are probably not valid in vivo. Both they and Bajec et al. (1958) found that indicator often failed to appear in the atrium in known cases of atrioventricular valvular incompetence, and sometimes appeared in the presence of normal valves. The fact that the position of the injection catheter tip in the ventricle influenced the quantity of dye regurgitating into the atrium indicated that uniform ventricular mixing was uncertain (Bajec et al., 1958; Marshall et al., 1958; Irisawa, Wilson and Rushmer, 1960). Dye dilution curves recorded after injections of dye at various sites in the right ventricle (Swan and Wood, 1953) or left ventricle (Callahan, Brandenburg and Swan, 1955) in patients with ventricular septal defects have also demonstrated that uniform mixing does not occur in either of the ventricles. Sinclair et al. (1960), however, found that the estimated regurgitant fraction in dogs was not dependent upon the site

of injection in the left ventricle using the multiple sampling indicator method.

The problem is further complicated by the fact that uniform atrial mixing by the end of diastole is also required for valid results, a dubious assumption (Swan et al., 1954, 1956; Silver et al., 1956; Sinclair et al., 1960). Moreover, where the dye curve is recorded at the aortic root, concentration fluctuates considerably with each ventricular ejection, making extrapolation of the downslope, and estimations of true curve area, a difficult procedure (Holt, 1956).

A similar multiple sampling principle for the estimation of aortic regurgitant flow has been employed by Armelin et al. (1960). They injected indicator into the aorta and measured its concentration in the left ventricle and a peripheral artery, using the formula:

$\frac{\bar{Q}_B}{\bar{Q}_F} = \frac{a_V}{a_F}$ = regurgitant fraction, where $\bar{Q}_B$ is the mean flow over the whole cardiac cycle from aorta to left ventricle, $\bar{Q}_F$ is the mean flow from left ventricle to aorta over a similar period, and a_V and a_F are the areas under the indicator dilution curves recorded from the left ventricle and femoral artery respectively. As in the similar equation used in assessing mitral incompetence, the fraction of injected indicator which regurgitates into the left ventricle must be representative of the fraction of the total forward flow of blood that regurgitates. Armelin et al., (1960) have shown no significant difference between the estimates of regurgitation by this technique, no matter whether the indicator injection

be carried out over the duration of systole, the duration of diastole, or over the whole cardiac cycle. Warner (1962) has explained theoretically that in all three cases an overestimate of regurgitant flow will result. If injection is of short duration (0.1 sec.) in early diastole, all the injected dye may be swept back into the left ventricle for detection as a_V, even in the presence of only mild aortic incompetence. With injection over the whole cardiac cycle, that fraction injected during diastole would be subject to the same error. With pansystolic injection the concentration of dye, expressed at the end of systole as a function of the distance down the aorta from the aortic valve, would not be uniform, but would depend upon the time course of flow velocity during systole past the injection site. The highest concentration of dye would be closest to the aortic valve, since flow velocity out of the left ventricle is maximal in early systole, and overestimation of backflow would therefore result.

(3) The third method of measuring backflow was devised by Lange and Hecht (1958 a, 1958 b). They injected dye into a peripheral vein and recorded simultaneously the concentration in the pulmonary artery and a systemic artery. The difference in appearance times and mean circulation times between the two curves was used to estimate the severity of the mitral regurgitation. This approach depended upon the relative smearing of dye distribution with passage through the pulmonary circuit and left heart, compared with the smearing effects of passage from a systemic vein to the pulmonary artery. In cases of combined mitral and aortic

regurgitation, by recording curves from the pulmonary artery, left atrium, and femoral artery, they claimed to be able to assess the degree of backflow for each incompetent valve. Marshall et al. (1958) believe that this smearing is largely determined by the volume of the left atrium and ventricle and not by the magnitude of the regurgitant flow, and cannot therefore be expected to yield direct information about the volume of backflow.

(4) Warner and Toronto (1958) devised an ingenious method for estimating the volume of backflow per stroke in aortic regurgitation. Repeated injections of dye were made into the descending aorta, each time increasing the distance of the injection catheter tip from the origin of the left subclavian artery. Dye curves were recorded from the left radial artery, and eventually a point was reached at which the distance travelled by the regurgitant dye during diastole was insufficient to enter the left subclavian origin and produce a registrable dye curve at the left radial artery. The calculated distance of the injection catheter tip from the left subclavian artery origin was multiplied by the approximate cross-sectional area of the aorta to represent the volume of blood regurgitating from this segment of the vascular bed per diastole. Unfortunately, the distance travelled by the farthest moving dye particles is not representative of the whole regurgitant blood column. Warner and Toronto (1961) have now shown that a tachycardia results in a much more drastic decrease in aortic regurgitation, as calculated by their technique, than could be explained by taking into account the change in the fraction of the cardiac cycle occupied by diastole at the increased heartrate, and by reasonable

assumptions regarding the inertia of the blood column. The progressive development of laminar flow during diastole causes an apparent marked increase in aortic regurgitation at slow heartrates. The technique can therefore be used only semiquantitatively, and the heartrate during the study must be taken into consideration (Warner, 1962).

Regional Blood Flows

The Problems associated with Regional Blood Flow Measurement with special Reference to the Measurement of Forearm Blood Flow: In the estimation of cardiac output by the dye-dilution method described mixing presented no problem, as the heart lay between the injection and the sampling sites and served as a mixing chamber, in addition to which turbulent flow occurred at the pulmonary arterial and aortic roots (Prec, Katz, Sennett, Roseman, Fishman and Hwang, 1949). In studies of regional flow, however, there is no guarantee of mixing due to naturally occurring turbulence, and the question therefore always arises whether estimates of flow are valid. It is appropriate to consider the conditions of mixing required for a valid measure of flow by indicator-dilution techniques, as outlined by Meier and Zierler (1954) and Zierler (1962b).

Consider a vascular system comprised of two common channels in which mixing occurs, with two randomized branching networks of vessels, as shown in Figure 29. The following injection and sampling sites should be considered in such a system:

(a) Injection at A,B, or C and sampling at D or E will give a valid measure of flow

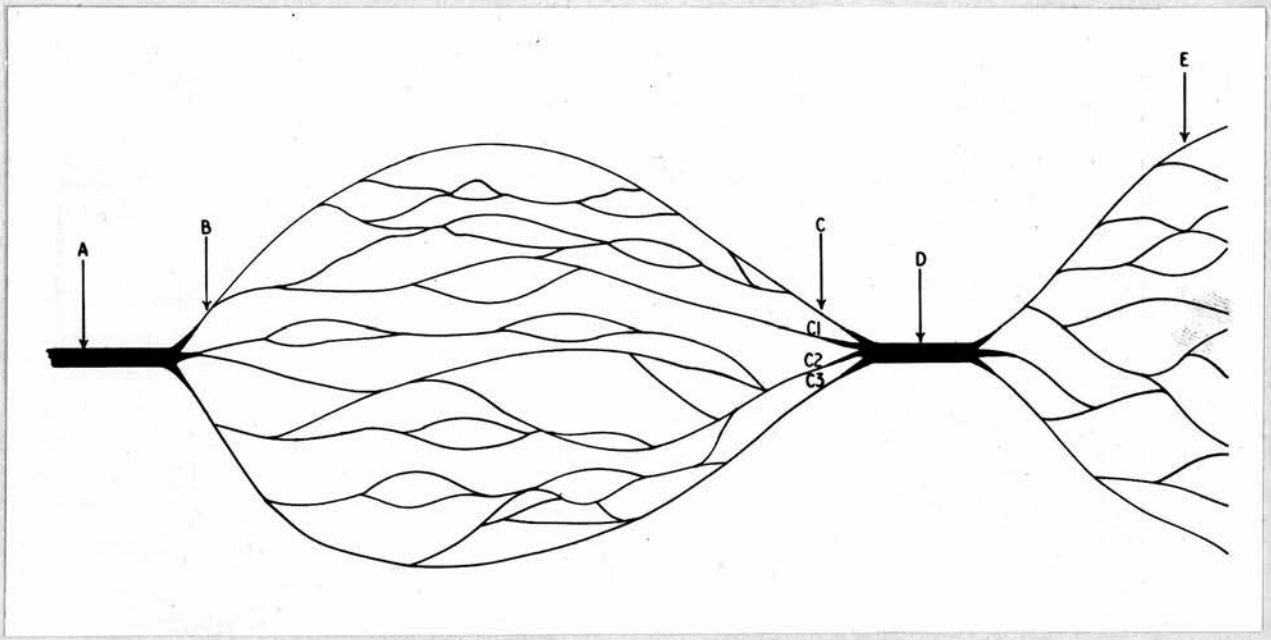

Figure 29: Theoretical vascular bed

(b) Injection at A and sampling at B, C, D, or E will give a valid measure of flow

(c) Injection at B and sampling at C will not give a true value for flow unless mixing is artificially induced in the channel joining B and C before it divides

(d) Injection at B and sampling at C may alternatively give a true measure of flow if fluid from each input channel mixes with that from every other input channel via a very rich system of vascular anastomoses before it leaves the system (Zierler, 1962b). To check that this has occurred, the time-concentration curve of indicator from every output channel (C, C_1, C_2, C_3) must be shown to be identical.

From the above theoretical considerations an idea can be obtained of the suitability of indicator-dilution methods for measuring flow in the various regional circulatory beds. The one feature common to all successful systems is a site where mixing will occur. The only sites in the body where turbulent flow is guaranteed, apart from the heart, are the pulmonary arterial and aortic roots. For valid estimates of flow elsewhere, therefore, artificial turbulence must be caused by a high injection velocity, sufficient to cause local turbulence, or there must be an extremely rich system of communicating vessels as in example (d).

A few attempts had been made to measure regional flow through the brain (Gibbs, Maxwell and Gibbs, 1947; Shenkin, Harmel and Kety, 1948) before Andres et al. (1954) performed their extremely thorough and comprehensive investigation into the problems involved in regional flow

studies by indicator-dilution techniques in an attempt to measure forearm blood flow. Their investigation covered almost all the problems which complicate the successful application of the technique and will therefore be used as an example to illustrate these various aspects.

As was explained in the earlier part of this section, mixing must be artificially created to satisfy the conditions for valid measurement of flow under certain circumstances. This very problem faced Andres et al. (1954) in attempting to measure forearm blood flow by injecting dye into the brachial artery and sampling from a forearm vein, since natural mixing was not sufficient. The ratio of forces tending to drive particles apart (inertial) to the forces tending to hold them together (viscous) can be stated for any specific flowing system as a dimensionless expression called the Reynolds number. When the former forces are sufficiently dominant, flow is turbulent and mixing occurs; otherwise flow is laminar. At the transition between laminar and turbulent flow, the ratio defines the critical Reynolds number. The critical Reynolds number for blood has been determined in vitro by Coulter and Pappenheimer (1949), and in vivo by Reynolds, Light, Ardran and Pritchard (1952). In vitro it is about 1000, and in vivo about double this value. To achieve turbulent flow in any vessel, it is immaterial whether turbulence originates by alteration of the Reynolds number of blood or of injectate, as in the vicinity of the site of injection the character of flow is a property of a new fluid system composed of both blood and injectate. Random intermingling of dye-laden injectate and

blood will occur if the Reynolds number of the injectate is sufficiently great to provoke a disturbance over the entire cross-section of the vessel in question. Andres et al. (1954) pointed out that the best method of increasing the Reynolds number was to increase the linear velocity of the injectate and to inject against the stream of flowing blood. They calculated the injection rate and jet orifice size necessary to obtain a suitable linear velocity to achieve turbulence in the brachial artery within a few centimetres of the site of injection. Provided that the necessary mixing was achieved before division of the vessel concerned, the method would therefore allow valid estimation of flow by the indicator-dilution technique. As mentioned earlier, adequacy of mixing could be verified by simultaneous sampling from more than one effluent vessel, and the demonstration of equal dye concentrations. By sampling from several veins they found, however, that their jet injector did not always achieve adequate mixing when forearm blood flow was increased. This highlighted yet another problem, namely that as the rate of blood flow increased, although not sufficiently to create naturally-occurring turbulence, the distance from the injection site over which mixing occurred was increased, and that inadequately mixed dye and blood could be swept into the branches of the main vessel, unlike at lower rates of blood flow where mixing was complete within a zone proximal to the bifurcation of a vessel.

In the particular situation of the forearm which they were studying, it transpired that mixing within the brachial artery before bifurcation was not essential to achieve a valid estimate of flow in about

80 per cent. of the subjects studied, because sufficient intermingling of blood originating from radial and ulnar arteries occurred to produce a relatively uniform distribution of dye among the veins draining the forearm, as was explained in (d) at the beginning of this section. Since this situation may not be present in other regional vascular beds, high velocity injection may be necessary to satisfy the conditions described earlier in (c). This, therfore, requires tailoring of the injection velocity to the rate of blood flow past the injection site, to ensure mixing of injectate and blood within the zone prior to bifurcation of the vessel. Far more important, however, is the fact that high velocity injections cause haemolysis, and that haemolysed blood has vasodilating properties (Chambliss, Demming, Wells, Cline and Eckstein, 1950; Folkow, 1953; Andres et al., 1954; Crowley, Grace, Fox and Wood, 1955; Grace et al., 1957; Nilsson, 1957). It was suggested by Fleisch (1937, 1938) and subsequently confirmed by Binet and Burstein (1950) that the vasodilator effects of haemolysed blood is due to the release of adenosine triphosphate and related substances from erythrocytes. Mechanical destruction of erythrocytes is related to the kinetic energy per unit time of the injection. Andres et al. (1954) calculated the critical injection velocities necessary to cause haemolysis and demonstrated up to ten-fold increases in total blood flow over the pre-injection resting values in experiments on the upper arm flow in man. Similar observations have been made in the lower extremities of the cat (Fleisch, 1937; Folkow, 1953), the hindlimb of the dog (Andres et al., 1954), the legs of man (Crowley et al., 1955;

Grace et al., 1957), the coronary circulation of the dog (Chambliss et al., 1950), and in the human brain (Nilsson, 1957). Nilsson (1960) suggests that this vasodilatation phenomenon may have contributed to the comparatively high flow values which have been found in some determinations of human cerebral blood flow by the injection of indicator into a carotid artery (Nylin and Blömer, 1955; Nylin and Hedlund, 1958). A recent study by Shillingford, Bruce and Gabe (1962) described the measurement of segmental venous blood flow by high velocity spray injection against the stream with sampling only seven millimetres downstream. Model studies suggested adequate mixing up to flow rates of four litres per minute in tubes of similar calibre to the veins being studied, and demonstrated accurate measurement of flow by the method. No mention is made, however, of the degree of haemolysis produced by injection at 3 ml./sec. through three jet orifices 0.033 cm. in diameter. Assuming the specific gravity of the injectate to be unity, this would produce a kinetic energy per second of injection of 683,280 g. cm.^{2} sec.$^{-2}$ per jet orifice, which is well above the lower haemolysing range of 10,000 to 20,000 g. cm.^{2} sec.$^{-2}$ suggested by Andres et al. (1954) in their studies where indicator was injected into the brachial artery.

It is apparent therefore that the production of artificial turbulence by a high injection velocity to obtain a valid estimate of flow in the situation described in (c) so alters the very flow which is being measured, that it may become quite useless in practice. In circulatory beds of this type, therefore, the only possibility of measuring flow

correctly rests with the situation described in (d), where a very rich system of vascular anastomoses causes mixing of blood from each input channel with that from every other input channel before it leaves the system. Andres and his colleagues were in fact able to measure flow in the forearm without resort to high velocity injection because the situation described in (d) exists in the average person's forearm.

With injections of low velocity into the brachial artery mixing was incomplete, and the radial and ulnar arteries received unidentical dye concentrations. The concentrations of dye in the two veins sampled differed significantly. Nevertheless, there was sufficient intermingling of venous blood for the two concentrations of dye to approximate each other. Any intermingling of venous blood had the effect of reducing differences in dye concentration, approaching that concentration which would have existed if dye had been distributed uniformly by arterial perfusion. If, for example, incomplete mixing in the brachial artery caused the radial artery dye concentration to be twice that of the ulnar artery, interchange of only one-third of the blood between the two veins, one draining exclusively ulnar, and the other exclusively radial arterial blood, would yield concentrations of dye in the two veins which differed from their mean by only $\pm$ 11 per cent. A mean concentration of dye was therefore considered to yield a valid measure of blood flow in about 80 per cent. of subjects. The exceptions, however, draw attention to other considerations necessary for the valid measurement of regional flow; the reasons for the exceptions were suggested by Andres' group as follows:

(i) Owing to anomalous high bifurcation of the brachial artery, dye was injected unknowingly into either the radial or ulnar artery, and venous intermingling was inadequate to produce randomization of dye concentration when one of the two major arteries to the forearm was completely free of dyed blood.

(ii) The rate of dye injection into the laminar flow of the brachial artery was so slow that a thin filament of dye-laden blood flowed almost entirely into either radial or ulnar artery, causing a situation similar to that in (i).

(iii) Some patients had vascular networks in their forearms which were not sufficiently luxuriant to produce randomization of dye particles.

(iv) There was asymmetric distribution of collaterals about the elbow carrying a major portion of forearm flow, so that ulnar and radial arterial blood were not diluted to an equivalent degree by the collateral inflows.

Exception (i) raises the problem of anatomical abnormalities invalidating flow measurements. Quain (1844) has shown in cadaver studies that bifurcation of the brachial artery occurs above the elbow in 20 per cent. of people. Palpation in the antecubital space seldom reveals two distinct arterial pulsations in such cases, as one of the divisions often lies deep to muscular or tendinous tissue, leaving only one arterial pulse palpable. In other instances it is likely that the two arteries are so juxtaposed that detection of separate pulsations is impossible. The only way to resolve this problem would be to perform

an arteriogram prior to measuring flow, and to exclude subjects with anomalous bifurcations from study. As will be seen later, similar problems arise in studies of renal blood flow.

Exception (ii) emphasizes that while injection velocity need not be high enough to produce true turbulence, it should be of sufficiently high velocity to disturb the laminar nature of the flowing blood pattern in order to produce some mixing.

Absolute injection velocities must therefore be chosen according to the anatomy of the bed under study. Very little is known at present about the Reynolds numbers existing in the various sections of the human vascular system (McDonald, 1960), and one has to work from assumptions. If the injection site is very near a downstream division of the vessel, it is especially desirable to achieve the maximum degree of turbulence than can safely be allowed without causing haemolysis. The velocity of injection necessary would obviously be less in an artery than in a vein, and in an artery with a right angled origin from the aorta such as the renal artery than the brachial artery in the region of the elbow.

Exceptions (i), (ii), (iii) and (iv) can all lead to non-uniform mixing. Exception (i) produces a situation in which flow may not be measurable by dye-dilution methods, and such a situation can be assessed by arteriography. How far exception (ii) can be overcome by a suitable injection velocity in any particular vascular bed can be ascertained by sampling from several effluent vessels where anatomically possible, and measuring the degree of equality of the dye concentrations

in each. Exceptions (iii) and (iv) can be assessed by the same multiple sampling technique, and unfavourable results will exclude such subjects from the study.

A problem which has not been investigated so far, is to what extent the catheters themselves interfere with the normal flow to the circulatory beds under study. A catheter may occupy a considerable proportion of the cross-sectional area of the vessel, and so obstruct adequate inflow or outflow of blood. Comparisons of flows by the dye-dilution method and another independent method over the same time period merely measure existent flow with the catheters in situ. To evaluate the degree of any interference with flow by the injection and sampling catheters, flow should be measured by an independent method before positioning the catheters to be used for the dye-dilution method, and repeated during the period of measurement of flow by the dye-dilution method. No reports have appeared so far in the literature of such an investigation having been performed in the study of any of the regional circulations.

Finally the problem of accessibility of the regional circulations should be mentioned. Catheterization of both the renal artery and vein is not an easy procedure, although image intensifiers now give far better radiographic definition, and have greatly reduced the difficulty of such a procedure. A similar situation pertains to the catheterization of the superior mesenteric artery in the measurement of splanchnic blood flow. To approach the portal vein splenic puncture is necessary.

Since this is a common procedure in radiographic assessment of the portal venous system, dye injections can be made at the same time to measure splanchnic blood flow.

Circulation times through regional circulatory beds tend to be shorter than those across the lesser circulation in the measurement of cardiac output. For this reason it is most important that the indicator used, if it be a dye, attains a stable spectral absorption before it passes through the detection instrument. This has been discussed in a previous section with reference to the available dyes, and indocyanine green has been shown to be adequate in this respect.

For the measurement of total flow through any vascular bed one of the essential requirements of the indicator is that it is not lost by excretion, diffusion or metabolism between injection and sampling sites. A dye such as indocyanine green is therefore quite unsuitable for splanchnic blood flow measurement due to its rapid uptake by the liver.

With the foregoing general considerations of the problems involved, in regional blood flow studies by dye-dilution techniques, the practicability of measurement of flows through specific regional circulatory beds will be considered.

Cerebral Blood Flow: Basically, the brain is supplied by two internal carotid arteries and two vertebral arteries. The blood from these four vessels mixes incompletely in the circle of Willis and is drained by two internal jugular veins. Shenkin et al. (1948) found

that an average of 66 per cent. of the blood from a single internal carotid artery drained by the internal jugular veins appeared in the ipsilateral internal jugular bulb, and 34 per cent. appeared contralaterally. Nylin, Silfverskiöld, Löfstedt, Regnström and Hedlund (1960) reported similar proportions. This anatomical situation does not satisfy any of the conditions discussed earlier for the valid measurement of flow by an indicator-dilution technique. If, however, the above simplified anatomical pattern of the cerebral circulations were true, and flow into both internal jugular veins were assumed to be equal, the mean of the indicator-dilution curves recorded from each internal jugular bulb might give a valid estimate of flow (Nylin and Hedlund,1958; Fox, Donald, White, Stanger and Wood, 1960). Shenkin et al. (1948) have shown that the problem is a more complicated one. The internal carotid arteries give off the ophthalmic arteries before supplying the brain, and they cannot readily be catheterized to a point beyond their origin. In fact, Nylin et al. (1960) comment on the difficulty of injecting sufficiently high up in the internal carotid artery to prevent regurgitation of indicator downwards into the external carotid artery. Important mixing of the blood of the brain and extracerebral structures occurs on the venous side of the circulation, and Shenkin et al. (1948) demonstrated that approximately 22 per cent. of blood in the external jugular vein is derived from the brain, and approximately three per cent. of the blood in the jugular bulb is derived from extracerebral structures. Moreover, the ophthalmic arteries are branches of the

internal carotid arteries and the eyes and their appendages drain principally into the cavernous sinus and thus to the internal jugular veins. Their flow would therefore be included in any measurement of cerebral blood flow. Although Nylin's group (Nylin and Blömer, 1955; Nylin, Blömer, Jones, Hedlund and Rylander, 1956; Nylin and Hedlund, 1958; Nylin et al., 1960) claim adequate mixing in the brain of indicator and blood, and that their values obtained represent absolute values for cerebral blood flow, their assumptions are too sweeping, and their theorizing unconvincing. It appears therefore that cerebral blood flow does not lend itself to measurement by indicator-dilution techniques because of the nature of the vascular anatomy.

Splanchnic Blood Flow: Bradley, Ingelfinger, Bradley and Curry (1945) were the first to devise a practical method of estimating splanchnic blood flow in man, using the principle of bromsulfalein (BSP) removal by the liver. This method, however, has the limitation of being suitable only for the determination of average flow over a minimum of ten minutes (Castenfors, Eliasch and Hultman, 1960), giving much the same limitation in this respect as the Fick method for cardiac output. Moreover, BSP extraction may be impaired by liver disease, thus affecting the reliability of the method. If feasible, indicator-dilution methods would therefore be of considerable value in both normal and diseased states, and during rapidly changing physiological conditions in the measurement of liver blood flow.

The liver has a dual input supply, the portal vein and the hepatic artery, and several veins draining it. If complete mixing occurred within the liver, indicator could be injected into either the portal vein or the hepatic artery beyond its gastric and duodenal tributaries, and sampled from any of the hepatic veins, and a valid estimate of flow would be obtainable. It could also be injected into the superior mesenteric artery, but this would cause considerable dispersion of indicator before reaching the sampling site as it would have to cross two capillary beds, and this might cause problems in separating recirculated indicator. Unfortunately, the hepatic artery is of very narrow calibre, and almost inaccessible to catheterization in the intact subject. The portal vein is similarly inaccessible to catheterization, but it can be approached indirectly via the splenic pulp by percutaneous splenic puncture. Reichman, Davis and Gorlin (1958) have measured splanchnic blood flow via splenic puncture and right hepatic vein catheterization, using radioactive iodinated serum albumin (RISA) as an indicator. Several problems of interest were encountered in their method.

They were aware of the necessary criteria for a valid measurement of flow, which were as follows:

(a) The indicator must remain within the vascular system, and must not be removed by the liver.

(b) No indicator must be shunted away prior to the intrahepatic circulation, as would occur in the presence of oesophageal varices.

(c) Indicator must mix with the dual blood supply of the liver prior to the site of final sampling.

(d) It must be possible to sample the blood leaving the liver as it leaves the area of complete mixing through to the first circulation.

There is fairly convincing evidence that RISA does in fact remain intravascular in transit through the liver, and is not removed by the liver itself (Finnerty, Tuckman and Buchholz, 1958). Hepatic blood flows are obviously erroneous in the presence of oesophageal varices, and these had to be excluded on other grounds. This proviso naturally excludes studies of a most interesting group of patients who suffer from portal hypertension.

They offered somewhat indirect evidence for the essential mixing of the indicator with the dual blood supply of the liver. While recording continuously the radioactivity from the right hepatic vein, they also performed surface counting with a scintillation probe counter over the liver. Their evidence for complete mixing of indicator before, or soon after entering the hepatic circulation was that suprahepatic counting (representing intrahepatic circulation) and hepatic venous sampling (representing posthepatic circulation) yielded essentially similar values for hepatic blood flow. Statistically there was no significant difference in the variance between the two groups as opposed to variance within the groups, implying that the samples were homogeneous and the results of the two methods of measurement did not differ. Splenic venograms

frequently raise doubts about partial portal vein obstructions due to the laminar nature of flow in the portal vein, and there is substantial evidence supporting the fact that adequate mixing does not occur in the portal vein (Brauer, 1963). Laminar flow prevents adequate mixing of indicator and blood before entering the liver, and it could therefore be argued that a disproportionate section of the injectate streamed into one lobe of the liver, from which the hepatic venous samples were taken, and that only a portion of liver blood flow was being measured by both the hepatic venous sampling and the suprahepatic counter. Similarly, their evidence for uniform sampling was demonstrated by the reproducibility of consecutive determinations of hepatic blood flow, and good agreement of flows determined simultaneously by suprahepatic and hepatic venous counting, and BSP. BSP flows, however, can hardly be regarded as simultaneous, as the period of measurement is far longer. Reproducibility of results in consecutive estimations does not prove uniform sampling, as incomplete mixing may cause a fairly constant disproportionate partitioning of indicator to various parts of the liver, each with a different venous drainage. A far more convincing method of demonstrating adequate mixing would have been to have sampled from a different hepatic vein each time, or preferably from more than one vein simultaneously, and to demonstrate that the curve areas from each vein were the same. Murray and Nebel (1959) injected indocyanine green via a catheter in a mesenteric vein with its tip in the porta hepatis in dogs. They withdrew blood via a densitometer from different hepatic veins during a steady state, and found similar values for flow. This suggested that there was

adequate mixing of dye and blood within the portal vein prior to its division, and that portal venous and hepatic arterial blood mixed in similar proportions in different sections of the liver.

Reichman et al. (1958) encountered problems in approximately 20 per cent. of subjects studied, due to partial extrasplenic injectate loss or subcapsular sequestration. By using a radioactive indicator they could detect this when it occurred by scanning over the spleen, and the method of calibration used did not allow this injection loss to interfere with the accuracy of flow measurement as long as a slow release of indicator did not occur from the sequestrated pool between the time of inscribing the curve and the calibration reading.

Ideally, a dye would be preferred as the indicator, for the reasons given in an earlier section. Ballinger and Bartone (1959) measured liver blood flows at laparotomy using indocyanine green injected into the portal vein in dogs and sampled via a surgically-created common hepatic vein. They were apparently aware that the dye concentration obtained from an hepatic vein might not be representative of all sections of the liver, but their common hepatic vein did not necessarily overcome this problem, as there is no evidence of mixing in this short venous segment. They were also aware of the removal of indocyanine green by the liver, but felt that this was small enough during one transit through the liver not to result in any errors in the measurement of flow due to loss of dye. Their evidence for this deduction is open to question. Murray and Nebel (1959) applied a correction factor in their estimated hepatic blood flows to

allow for an hepatic uptake of 0.015 mg. indocyanine green per kg. body weight. This would have to be chosen according to liver function, where studies were performed on patients with liver disease. Reichman et al. (1958) have reported that Evans blue is not recoverable completely in hepatic venous blood after portal vein injection because of loss to the hepatic tissues, due to the incomplete nature of the binding of the dye to albumin so soon after its injection into the portal vein (Hyman, Bernick and Paldine, 1955). Coomassie blue would therefore appear the most suitable dye available for studying liver blood flow, as it does not diffuse into the liver in measurable amounts (Taylor and Shillingford, 1959).

Reversion to the use of the blue dyes, however, means that continuous recording methods will be interfered with by any sudden change in hepatic venous oxygen saturation. The choice of dyes therefore appears to lie between indocyanine green with an assumed correction factor for hepatic removal (Murray and Nebel, 1959) or coomassie blue using intermittent sampling techniques.

Two interesting applications of indocyanine green and indicator-dilution techniques have been described with reference to liver disease. If indocyanine green is injected into the spleen, and its time-concentration curve recorded at the right atrium or a systemic artery, its appearance time can give valuable information concerning the presence of portal-systemic shunts. Long and Lombardo and their colleagues (Lombardo, Long, Braunwald and Morrow, 1959; Long, Lombardo, Braunwald and Morrow, 1959) have shown considerable differences in the appearance time

and peak concentration of such curves in dogs when a surgically-created side-to-side portacaval anastomosis was clamped or released. Kr^{85} monitored from the expired air with a Geiger-Müller tube inserted into the airway, provided similar dilution curves, which were more sensitive, giving more complete separation of values obtained with and without shunts. The reason for this is that the Kr^{85} does not traverse the liver capillary bed as a simple intravascular indicator like indocyanine green, but diffuses into the intercellular and interstitial compartments before returning to the circulation. In the absence of a portal-systemic shunt its appearance time in the expired air is therefore markedly delayed. Moreover, it does not require right atrial catheterization or arterial puncture. Results suggest that the method will help to distinguish patients with a normal portal circulation, and those with oesophageal varices without surgical shunts, and those with varices and patent portacaval anastomoses.

The other application of indocyanine green in liver disease has been its use in a similar fashion to BSP for the measurement of liver blood flow (Wheeler et al., 1958; Hunton, Bollman and Hoffman, 1960; Ketterer et al., 1960; Reemtsma et al., 1960; Winkler and Tygstrup, 1960; Caesar et al., 1961). Since this is not an indicator-dilution technique, it does not merit further discussion in this text.

Renal Blood Flow: The normal kidney is supplied by one renal artery and one renal vein. There is no proof of turbulence in either vessel, but a rich system of communicating vessels within the kidney

probably allows sufficient mixing of blood to fulfil the criteria for a valid measurement of flow by dye-dilution techniques. In most other regional circulatory beds this could be checked by simultaneous sampling from several of the effluent veins, as described for splanchnic blood flow, but, since there is normally only one renal vein, this is not possible. Severe non-uniformity of mixing would, however, distort severely the dye-dilution curve recorded from the single renal artery due to rapidly fluctuating concentrations of dye from independent circulations within the kidney presenting themselves in rapid succession at the catheter tip. The dye-dilution curve recorded from the renal vein shows the smooth contour to be expected in the presence of adequate mixing, as will be demonstrated in a later section. Some degree of mixing is desirable in the renal artery, as was discussed in the problems associated with the measurement of forearm blood flow, for if all the injected dye streamed into one division of the renal artery, even the rich capillary network of the renal parenchyma might not be sufficient to overcome absolute lack of mixing at the site of injection in the renal artery. Although the degree of turbulence in the renal artery is as yet unknown, it is reasonable to assume that the nature of its right-angled origin from the abdominal aorta would cause considerable disturbance of flow pattern and thus a fair degree of mixing (McDonald, 1960).

Unfortunately, anatomical abnormalities are frequent in the vascular supply to the kidney. More than one renal artery would present a situation analagous to that of injection into either radial or ulnar artery

alone in the measurement of forearm blood flow discussed previously. Boijsen (1959) found an incidence of 23.8 per cent. of multiple renal arteries in persons with otherwise normal kidneys, and the incidence in congenitally abnormal kidneys is even higher. These supplementary arteries usually supply as much as 20 to 50 per cent. of the renal parenchyma, and in 20 per cent. of such cases the supplementary artery is of the same width as the main renal artery (Edsman, 1957). Arteries of this size would almost certainly show on angiography and renal blood flows obtained in such subjects could be rejected.

A further consideration in using dye-dilution methods for measuring renal blood flow is that the renal artery frequently gives off branches to surrounding structures such as the adrenal gland, renal capsule and ureter. The frequency of vessels supplying these structures arising from the renal artery itself varies between 39, 91 and 82 per cent. respectively (Boijsen, 1959). While these structures need very little blood relative to the flow to the renal parenchyma, non-uniformly dyed blood may escape via these vessels and not be mixed in the rich anastomotic network of the renal parenchyma.

The calibre of the renal vessels is not great in relation to the catheters necessary to enter them, and interference with blood flow probably occurs due to their presence. No studies of the effect of this have been reported in the literature.

It is of theoretical interest only to relate that a dye such as indocyanine green, which attaches itself to serum albumin, is non-representative of the total blood flow through the kidney (Zierler,1962b).

Since water leaves the renal vascular system between injection and sampling sites, indicator is therefore concentrated. As all the water does not return to the vascular system before sampling, the concentration of indicator in the renal vein blood represents renal venous and not arterial blood flow.

The use of dye-dilution techniques for the measurement of total renal blood flow is therefore inappropriate by virtue of the reasons discussed. In the absence of multiple renal arteries, however, they can consistently measure a constant fraction of flow very closely approximating that of total renal blood flow in the same subject. Flow can be measured over a far shorter time period than by clearance methods such as those using para-aminohippurate (Smith, Finkelstein, Aliminosa, Crawford and Graber, 1945), and rapidly induced changes in flow can therefore be assessed. No reports of the use of such a technique have been described in the literature, but a study conducted in this laboratory described later will illustrate that no other method available at present would have been suitable for the measurement of the rapid changes in renal blood flow produced.

Coronary Blood Flow: The method of measuring coronary blood flow by indicator-dilution techniques involves a somewhat different principle to that of other regional flows. At a constant flow, the ratio of two different amounts of injected dye ($\frac{Ia}{Ib}$) equals the ratio of the areas of the two different dilution curves to which they give rise ($\frac{Aa}{Ab}$) (Levinson, Cudkowicz and Abelmann, 1959). If the curve areas and

one of the injection amounts is known, the other injection value can be calculated:

$$\frac{Ia}{Ib} = \frac{Aa}{Ab}$$, and thus:

$$Ib = \frac{Ia.\ Ab}{Aa}$$

If a curve were inscribed, therefore, from the combined venous drainage of the coronary circulation during a normal dye-dilution curve for determination of cardiac output, the ratio of its area to that of the curve obtained from the aorta for cardiac output would be the same as the ratio of the total dye injection to the fraction of the injection entering the coronary arteries. The fraction of the injected dye circulating through a regional bed such as the coronary circulation on its first passage is therefore equal to the fraction of the cardiac output flowing through that regional bed. Although the coronary flow curve is generated from a non-instantaneous, more dispersed dye injection than that giving rise to the cardiac output curve, the area relationships hold, despite the distortion of the coronary flow curve.

If the anatomy of the coronary circulation were suitable, coronary sinus catheterization would give access to the venous outflow curve of the coronary circulation. Unfortunately, the coronary sinus drains only part of the coronary circulation, and inadequate mixing of the various parts occurs prior to the coronary sinus. Moreover, the technical difficulties of inscribing an indicator-dilution curve from the coronary sinus are considerable (Forte, Schmitthenner and Neal, 1961).

Attempts were originally directed at constructing the coronary circulation curve from the recirculation hump of the original cardiac output curve (quoted by Conn, 1962). This implied that the recirculation hump was entirely due to coronary recirculation. Because the upstroke of the recirculation hump was ill-defined, additional right ventricular or pulmonary arterial sampling was performed to obtain the recirculation curve. Huff (quoted by Conn, 1962) found that the recirculation curves so obtained were not due to coronary recirculation alone, but were a mixture of thyroid, bronchial and coronary flow. Subsequent work by Marchioro and co-workers (Marchioro, Owen, Lester, Montgomery and Swan, 1959; Marchioro, Feldman, Owen, and Swan, 1961) also pointed to the unreliability of the method for the same reasons as found by Huff.

Brief mention should be made of radioactive indicator-dilution methods of measuring coronary blood flow by surface counting, although they do not fall within the adaptability of the dye-dilution method described in this study. The principle is the same as that described using a dye, in that coronary blood flow is related to cardiac output as the fraction of injected indicator passing through the coronary circulation to the total injectate. Measurement of praecordial radioactivity, however, is substituted for measurement of indicator concentration in the right heart, and an additional variable of the relative mean velocity of blood flow past the myocardial and heart chamber volumes being scanned by the detector must be taken into consideration (Sevelius and Johnson, 1959). Bing, Hellems and Regan (1960) and Conn (1962) have outlined the many problems associated

with the technique, which suggest that it is far from satisfactory. They describe equally many shortcomings in the methods of Love and Burch (1957), and Nolting, Mack, Luthy, Kirsch and Hogancamp (1958), of praecordial measurement of Rb^{86} uptake by the myocardium as an index of coronary blood flow. It appears, therefore, that no absolutely satisfactory method of measurement of coronary blood flow by an indicator-dilution technique is yet available.

GENERAL DISCUSSION

Methods for the Future

The Dichromatic Densitometer: It has been repeatedly mentioned in the course of previous sections how the advantages gained by the use of indocyanine green were to a certain extent outweighed by reversion to monochromatic densitometry. The reason for this was given as the interference with the recording of dye concentration by nonspecific optical density changes in blood. Sinclair et al. (1960, 1961) have demonstrated these effects very clearly using the monochromatic densitometer, while Sutterer (1960) and Wood (1962b) have shown equally convincingly that the new dichromatic densitometer can overcome these effects. They are not purely due to haemodilution, as the addition of isotonic solutions to blood produce very small optical density changes, whereas the addition of identical volumes of hypertonic solutions cause large increases in optical density. The changed osmolarity of plasma apparently alters the shape and volume of the erythrocytes, which therefore changes the reflection, transmission and refraction of incident light by the cells, and so affects the complicated phenomena of their light absorption (Read et al., 1960). Sinclair et al. (1961) therefore suggest that an increase in plasma osmolarity causes the erythrocytes to shrink, and their highly curves surfaces reflect more light, while less is transmitted, and so the optical density of blood is increased. As has been discussed previously, changes in the rate of blood flow through the cuvette can cause considerable,

largely unexplained fluctuations in optical density, but Sinclair et al. (1961) have shown that changes in pCO_2, which may well occur during patho-physiological studies, also cause interference. In studies on dogs, artificially ventilated with 100 per cent. oxygen, four breaths of 50 per cent. carbon dioxide in oxygen caused a peak negative deflection of 6.3 cm., compared with a peak positive deflection of 24.5 cm. caused by an injection of 2.5 mg. of indocyanine green solution in the same animal. This phenomenon is unexplained, but other workers have provided evidence that changes in temperature (Jacobs et al, 1936; Pappenheimer, 1941) and pH (Brown, 1956) influence the transmission of light by their effects on theerythrocytic membrane, while urea affects optical density by its haemolytic action (Pinter and Zilversmit, 1960).

These difficulties were minimized before the advent of indocyanine green by the dichromatic oximeter circuit. The effect of changes in light transmission at the wave-length at which the dye was maximally absorbed was bucked against the effect of changes in light transmission at another wave-length, absorbed to a different degree or not at all by the dye, but affected to the same degree by nonspecific optical density changes of the blood. Changes in optical density occurring simultaneously at both red and infrared spectral regions were therefore cancelled out.

With the monochromatic densitometer (Waters XC-250A), as used in this study, the sources of the errors discussed were avoided, and the main criticism lies with the pulsatile traces. An instrument which will overcome almost entirely the effect of these nonspecific optical density

factors, will be almost insensitive to flow through it, and which will produce nonpulsatile curves, is the dichromatic densitometer designed by Sutterer (1960) (Figure 30). Light passes through the blood sample to a dichroic mirror fixed in position so that the angle of incident light on the mirror face is 45 degrees. The mirror's spectral characteristics are such that it reflects light in the region of 800 mμ to a dye-detecting photocell, via a filter used for accurate adjustment of photocell optical sensitivity, while it transmits light of wave-lengths longer and shorter than 800 mμ to a second compensating photocell, via another filter. Figure 31 shows the spectral sensitivity of the two photocell-filter assemblies used in the dichromatic densitometer, and the spectral transmission of oxyhaemoglobin and reduced haemoglobin. The compensating photocell-filter assembly will be seen to have a minimal sensitivity to wave-lengths in the region of 800 mμ, maximally absorbed by indocyanine green, and peaks in sensitivity on either side of this wave-length band, while the dye-detecting photocell assembly has its peak sensitivity at 800 mμ. Insensitivity of this compensating photocell output to changes in oxygen saturation and dye concentration is attained by proper adjustment of the relative sensitivity of the compensating photocell on the two sides of the isosbestic point.

This new instrument is no less sensitive to indocyanine green than the monochromatic densitometer, and Sutterer (1960) and Wood (1962b) have shown that their peak deflections are similar for equal dye injections. Deflections following the injection of equal volumes of 10 per cent., however, were 5.0 cm. for the monochromatic instrument (Waters XC-100)

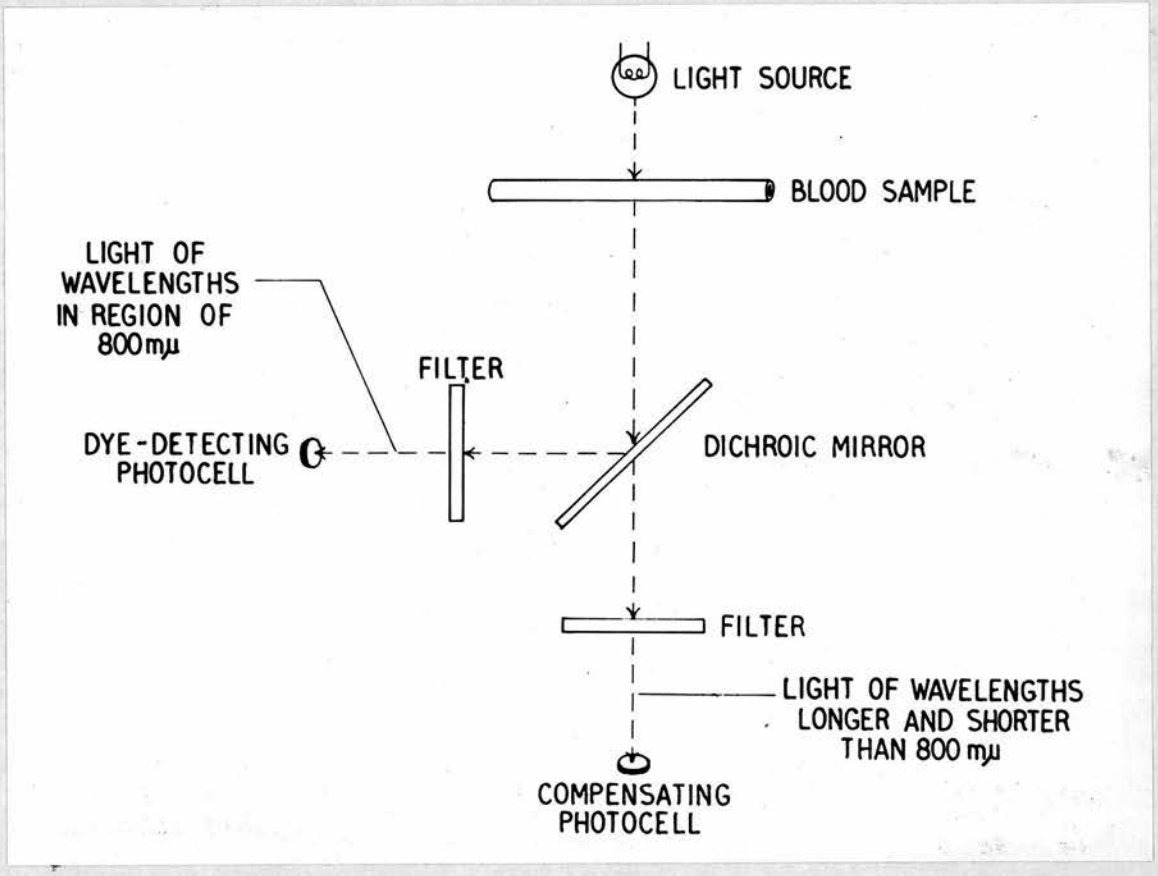

Figure 30: Photocell-filter assembly of dichromatic densitometer (Fox,1962b)

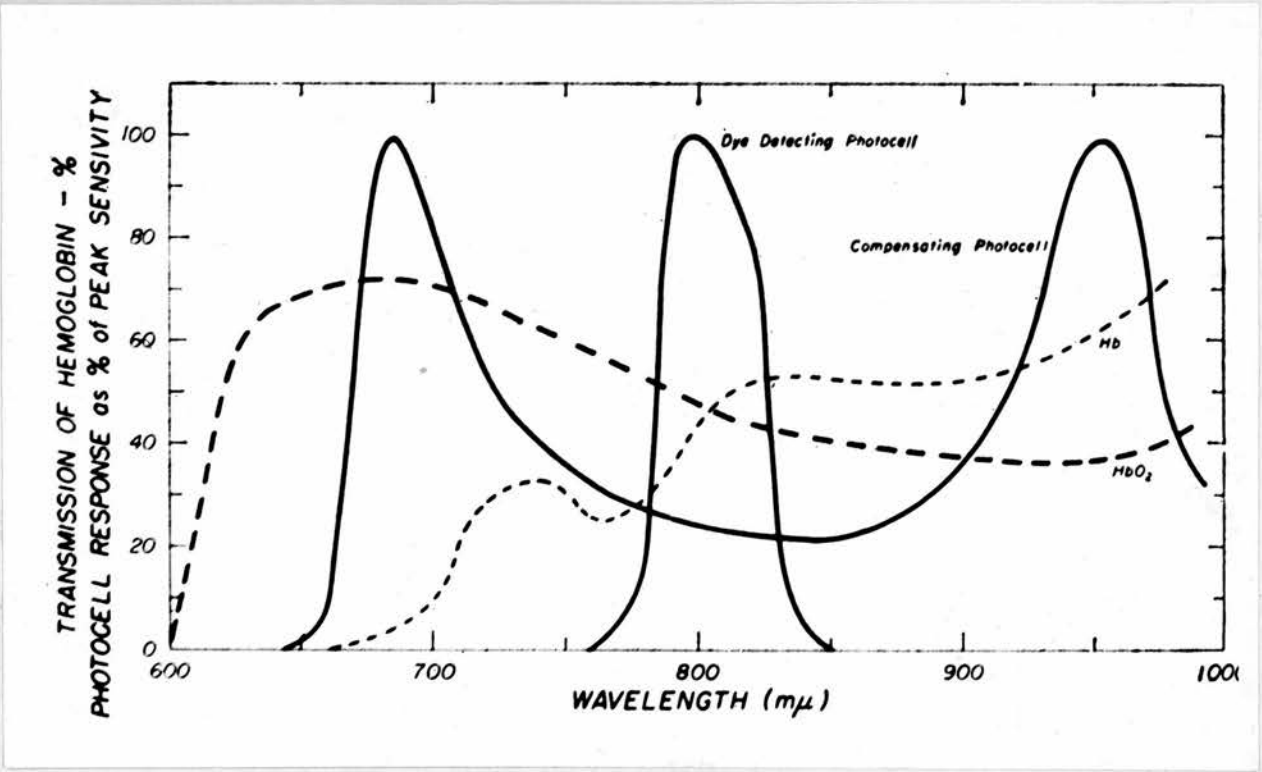

Figure 31: Comparison of spectral sensitivity of the two photocell-filter assemblies used in the dichromatic densitometer for indocyanine green, and the spectral transmission of oxyhaemoglobin and reduced haemoglobin (Wood,1962b)

as against 0.6 cm. for the dichromatic densitometer (Waters XC-300). Similarly, sudden cessation of flow through the instruments caused deflections of 4.5 cm. and 0.5 cm. respectively.

The Intravascular Catheter-Tip Densitometer: Perhaps the most promising approach to attaining high fidelity recordings of dye-dilution curves from sites in the central or regional circulations in man is the development of a catheter-tip densitometer for use with indocyanine green. Dr. Michael Polanyi, of the American Optical Company, and his associates have developed a catheter-tip oximeter based on the reflection principle, and have avoided the need for extreme miniaturization by the use of fibre optics to transmit the incident and reflected light beams from the external end of the catheter to and from the blood flowing past the tip (Polanyi and Hehir, 1960; Enson, Briscoe, Polanyi and Cournand, 1962). A two-colour or dichromatic assembly is employed to compensate for the nonspecific effects on the reflectance of blood, caused by variations of blood flow past the catheter tip. The output fibre optical channel is split, to view the reflected light from the same blood surface within the circulation at two wave-lengths, one specifically affected by indocyanine green, and the other specifically selected to act as a baseline for the inscription of the curve, unaffected by the dye, and to compensate for pulsatile flow. Figure 32 shows a diagrammatic representation of the instrument. Two rotating optical filters with peak transmissions at 806 mμ and 900 mμ traverse the path of the incident light alternately, 40 times per second, before it passes via an optical

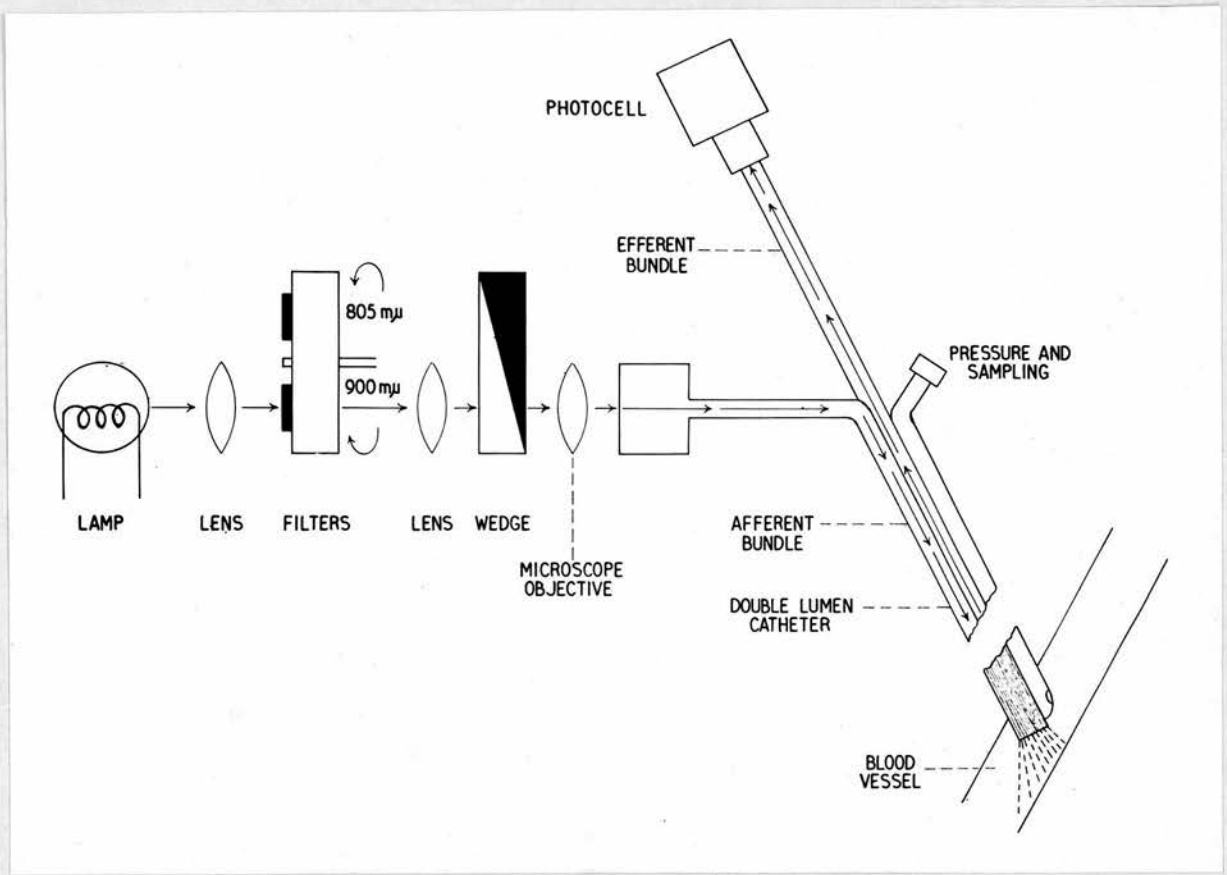

Figure 32: The intravascular catheter-tip densitometer (Enson et al., 1962)

wedge to the glass fibres, and so to the bloodstream. Here the light is absorbed, refracted, transmitted, and diffusely back-scattered. This diffusely "reflected" light is conducted back outside the body via an identical set of fibres, contained within the same lumen of the catheter, to a photomultiplier.

The instrument described is potentially a great advance in dye-dilution techniques; it removes all hydraulic factors which cause so much distortion of curves, and it requires no blood withdrawal during the procedure. The present prototype, however, has two disadvantages. One is that the relationship between dye concentration and the ratio of intensities of reflected light ($\frac{I_{R900}}{I_{R805}}$) is not linear above 10 mg./l. of indocyanine green in blood. This can be overcome by limiting the amount of dye per injection to some extent, although the instrument cannot discriminate in vivo concentrations of less than 0.28 mg./l. (Enson et al., 1962). The other drawback, a serious one, is that apparent dye concentration is affected by the oxygen saturation of the blood, especially at low saturations. A 10 per cent. rise from 85 to 95 per cent. saturation results in an underestimation of dye concentration by about two per cent. while a 10 per cent. rise from 40 to 50 per cent. saturation results in an underestimation of dye concentration by about 15 per cent. of its real value (Enson et al., 1962). At present, efforts are being made to overcome this problem by the use of a "pseudo-isosbestic" filter, an interference filter with peaks at 660 mμ and 900 mμ, as a baseline to render the system insensitive to changes in oxygen saturation (Sutterer and Polanyi, Unpublished data).

The Use of Computers in the Problem of Curve Distortion and the Calculation of Cardiac Output: In a previous section the measures taken to minimize distortion of dye curves were discussed. All measures, however, were merely improvements, not providing complete answers, and further improvements would require rates of blood withdrawal far too great, in order to give sufficiently rapid dynamic response to follow the variations in indicator concentration of the frequencies encountered in the central circulation.

Recently, attempts have been made to recover the dye curve as it really appears at the catheter tip, by a numerical recursive method of calculation, based on the curve recorded by the system, and the measured response of the system to a stepwise change in indicator concentration (Cheesman et al., 1959; González-Fernández, Cheesman and Wood, 1959). The technique for recovery of "true" dilution curves is not practical for widescale application, since it requires an extremely large number of accurate measurements of the recorded curves, followed by extended arithmetical calculations involving solution of an integral equation of the convolution type.

The use of electronic data-handling processes in conjunction with analogue computer techniques have made this practicable. González-Fernández et al. (1959) have successfully employed a general-purpose digital computer, while an analogue-digital conversion system can reduce the measurement time to a matter of seconds. Figure 33 is an illustration of how effective this system is. Dye curves were inscribed simultaneously by two catheter-densitometer systems with a slow and a fast dynamic

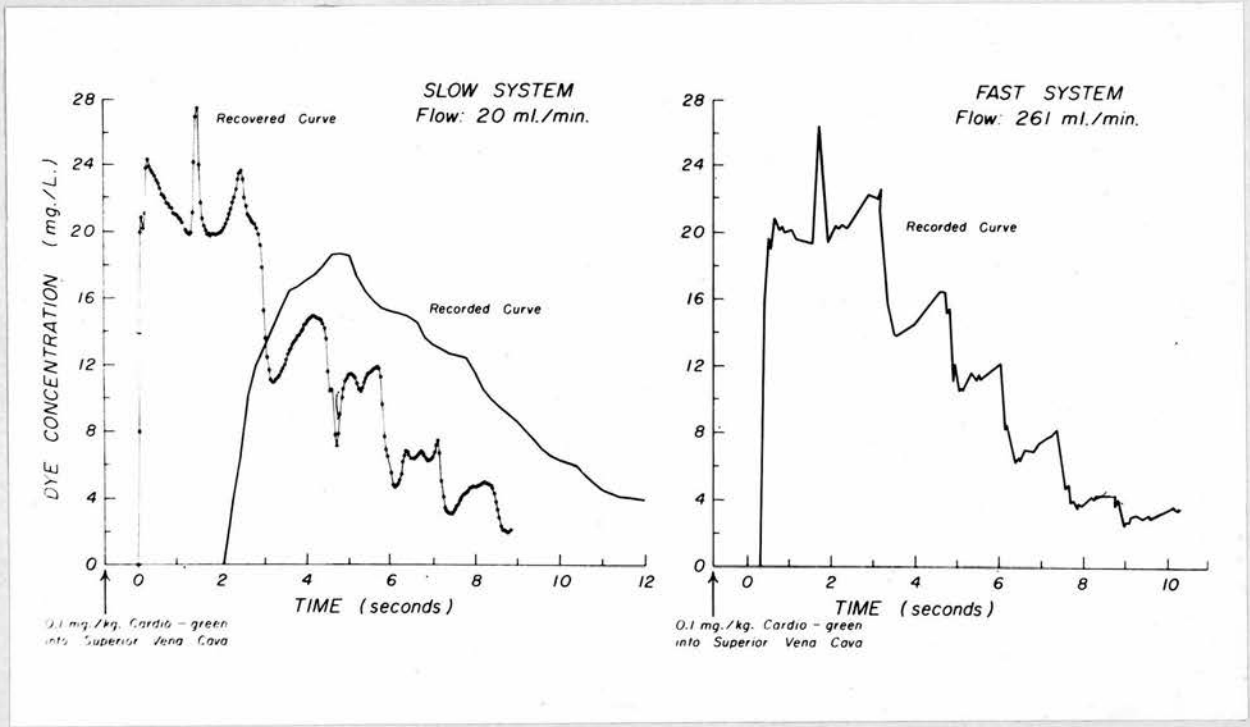

Figure 33: Comparison of dye-dilution curves recorded simultaneously from the same site in the pulmonary artery of a dog by two catheter-densitometer systems (one with a slow and one with a fast dynamic response) with "true curve" at catheter tip recovered mathematically (Wood, 1961)

response respectively, sampling from the pulmonary artery of a dog. The mathematically recovered "true" curve is also plotted, and it can be seen just how closely it resembles the true curve, obtained at the impractically high withdrawal rate of 261 ml./min. (Wood, 1962b).

The process of calculating the area of a dye curve involves the time-consuming process of careful measurement at fixed time intervals, a semilogarithmic replot, and a lengthy arithmetical calculation. In a busy laboratory large numbers of dye curves may be produced per week. To obtain a value for cardiac output alone from such curves takes a skilled operator approximately 15 to 25 minutes per curve. In terms of analysis, therfore, the estimation is about as time-consuming as a Fick analysis.

In 1961, the first reports of computer analysis became available. Danielson, Summers, Norman and Blakemore (1961) described a portable digital computer giving an answer for cardiac output in litres per minute 30 seconds after completion of the first circulation of the indicator. The computer was composed of two units, the first one computing the area under the curve up to a point on the downslope selected by visual observation, and the second computing the exponential portion of the downslope. The computer was precalibrated with quantitative information, obtained by a routine calibration procedure, in order to give direct readings of cardiac output. Model studies showed reproducibility of repeated determinations with a standard deviation of $\pm$ 5 per cent. of the mean. In dogs whose pulmonary artery blood flow was checked by an

electromagnetic flowmeter, cardiac output values checked with a standard deviation of ± 8 per cent. of the mean flowmeter values, which varied from 200 to 1500 ml./min.

The University of Saskatchewan has developed a computer which is now being manufactured commercially by the Waters Corporation. The computer gives a readout of cardiac output in litres per minute as well as mean transit time, and the error between computer and calculated values for cardiac output determinations is within seven per cent. (Merriman, Personal communication). The Gilford Instrument Laboratories Inc. have also recently made available a computer for the calculation of cardiac output, but the unpublished results so far show errors far greater than those using the Waters Computer (Manufacturer's brochure).

Eventually, therefore, the ultimate in dye-dilution techniques would appear to be an improved intravascular reflection densitometer, employing a "pseudo-isosbestic" filter, linked with a computer for calculation of cardiac output. Accurate cardiac output estimations would then become more freely accessible, and would no longer be the preserve of the specialised laboratory.

The Use of Diffusible Indicators: It was discussed in an earlier section that one of the essential properties of any dye used in indicator-dilution measurements of flow was that it should remain strictly intravascular between injection and sampling sites. This absolute rule applies to any indicator being used to measure flow. If, on the other hand, another substance were injected with the dye and were removed from

the bloodstream by the organ whose flow was being measured, its concentration-time curve, taken in conjection with the simultaneous dye concentration-time curve, would give an index of the removal by the organ of the substance in question. It would, in addition, give an indication of the volume of distribution of the indicator.

If a dye, such as Evans blue or indocyanine green, were injected into the pulmonary artery and sampled downstream to the lungs, its volume of distribution would be that of the intravascular blood volume with which it mixes between injection and sampling sites. If, however, another substance such as deuterium hydrogen oxide were injected, its distribution volume would be the intravascular blood volume between injection and sampling sites, plus the volume of the pulmonary liquid phase involved in gas exchange in the lungs with which it mixes. Simultaneous instantaneous injections, therefore, of the two indicators would give concentration-time curves with greatly different distribution volumes. Generally, and with a closed system, the greater the volume of distribution, the later and smaller the peak concentration will be, but the curve areas will be equal after correction for recirculation. If, however, indicator also reaches the gaseous phase, in other words the system is not closed, some is lost via respiratory exchange, and the area of its concentration-time curve in arterial blood is less than that for the reference substance.

Chinard, Enns and Nolan (1962) have devised a mathematical conversion to provide a simple and rapid method of assessing the relative distribution

of different indicators injected simultaneously, and of indicating the significance of the several possible phases of distribution. This is achieved by plotting the cumulative concentration-time curves on a logarithmic time base after the manner suggested by Stow and Hetzel (1954), and by transformation of the resultant regular sigmoid into a more or less straight line by the probit transformation (Finney, 1952). They found that the probit plots differed markedly between the reference substance (a non-diffusible indicator such as Evans blue), diffusible substances, and substances which distributed themselves in more than one compartment.

Using the above methods, Chinard, Enns and Nolan (1960) were able to calculate the fraction of labelled water which appeared to go into a greater volume of distribution than the reference substance, and showed that roughly half the water pumped across the lungs by the heart leaves the intravascular compartment and returns in the time of a single passage through the lungs. They were also able to show the increase in the volume of distribution of heavy water caused by the excess interstitial fluid present in pulmonary oedema.

Studies have also been carried out with different indicators in vivo to determine the extent to which dissolved carbon dioxide contributes to expired carbon dioxide, relative to the other forms in which carbon dioxide may exist, before and after interference with carbonic anhydrase activity by administration of acetazolamide (Chinard et al., 1960). Evans blue, labelled water, $C^{13}O_2$ dissolved,

and either $C^{14}O_2$ dissolved or $HC^{14}O_3^-$ were used as indicators, and anaerobic sampling of arterial blood and expired air were performed. They were able to show a disproportionately large contribution from dissolved carbon dioxide after inhibition of carbonic anhydrase, and that under these circumstances peripheral arterial blood is not representative in composition of end-alveolar capillary blood, due to failure of equilibrium distribution of gases between liquid and gas phases in the time of transit of blood through the alveolar capillaries.

Studies with inert gases such as T_2, Kr^{85}, Xe^{133} and C^{14}-labelled ethylene, which have differing diffusion coefficients and solubilities, have also been performed, to assess whether equilibration of inert gases between pulmonary capillary blood and alveolar air occurs within the time of transit of blood through the lungs, or whether there is a solubility or diffusion limitation (Chinard, Enns and Nolan, 1961). To assess the element of diffusion difficulty injections of the indicators in solution were made into the right heart, and to assess solubility difficulties tracheal injections of the gases were made, while arterial sampling was performed in both cases. Speed of diffusion and relative solubilities were assessed by the relative overall recovery in arterial blood of the gases concerned.

Chinard, Taylor, Nolan and Enns (1959) have injected a solution containing Evans blue, creatinine and labelled glucose via a catheter into the renal artery and sampled via another catheter from the renal vein in studies on dogs. They have measured the recovery of the glucose

and creatinine, assuming absolute recovery of the reference indicator, Evans blue, and were able to ascertain from their results the rate of glucose breakdown by the kidney, and its contribution to renal carbon dioxide production. Further experiments using labelled D and L - lactate and pyruvate indicated that a high proportion of renal carbon dioxide production comes from the carboxyl carbons of lactate and pyruvate, and that they are in fact the major immediate source. Their results also cast considerable doubt on the standard in vivo substrate-utilization methods, calculated as the product of blood flow and arterio-venous concentration differences.

Many of these techniques are limited at present by the necessity for the use of intermittent sampling techniques, because instruments are not yet available to detect the concentrations of substances such as creatinine continuously in flowing blood. The use of radioactive-labelled substances, however, lends itself to continuous recording, and detection instruments could be arranged in series giving recordings of the reference substance and the radioactive-labelled substrates being studied. Unfortunately, the use of radioactive-labelled substances immediately brings with it all the associated disadvantages such as dosage limitation, as discussed in an earlier section. It may well be that conductivity methods of continuous recording may find a place in these studies, as many of the substances of interest cause changes of electrical resistance in the blood. If the problem of adequate and uniform mixing at the injection site could be overcome, much information

of value could be obtained about liver metabolism, but, until this time, such techniques are impractical due to probable non-uniform indicator distribution, as discussed in an earlier section. Nevertheless, although studies with diffusible indicators are at present in their infancy, the information provided by the work described above is of considerable interest, and it would seem likely that these methods will provide a new and valuable chapter in indicator-dilution work.

2.4 mm. was inserted by a Seldinger technique into the right femoral artery. The catheter had been previously moulded so that its terminal inch formed an angle of approximately 70 degrees with the rest of its length. This catheter was manipulated under fluoroscopic control into the left renal artery. A 125 cm. 8 F single lumen catheter (United States Catheter and Instrument Corp.) was then inserted under local anaesthesia into the right antecubital vein and passed into the left renal vein in a similar fashion.

The concentration of indocyanine green injected was calculated to give curves of suitable dimensions while the peak concentration remained within the linear range of the system (< 25 mg./l.) (Figure 34). Because of the longer sampling catheter necessary, the suction rate was reduced to 19 ml./min. Trial curves indicated the appropriate sensitivity and paper speed. The subject was then required to grip the dynamometer at 20 per cent. and then 50 per cent. of his maximum, allowing suitable control periods before and after each contraction, and during all periods dye curves were inscribed at one-minute intervals from the left renal vein.

Results

Figure 34 shows one of the dye curves obtained. It will be seen that it has a smooth contour, suggesting that a uniform dyed blood mixture was presented to the sampling catheter tip. The curve returns

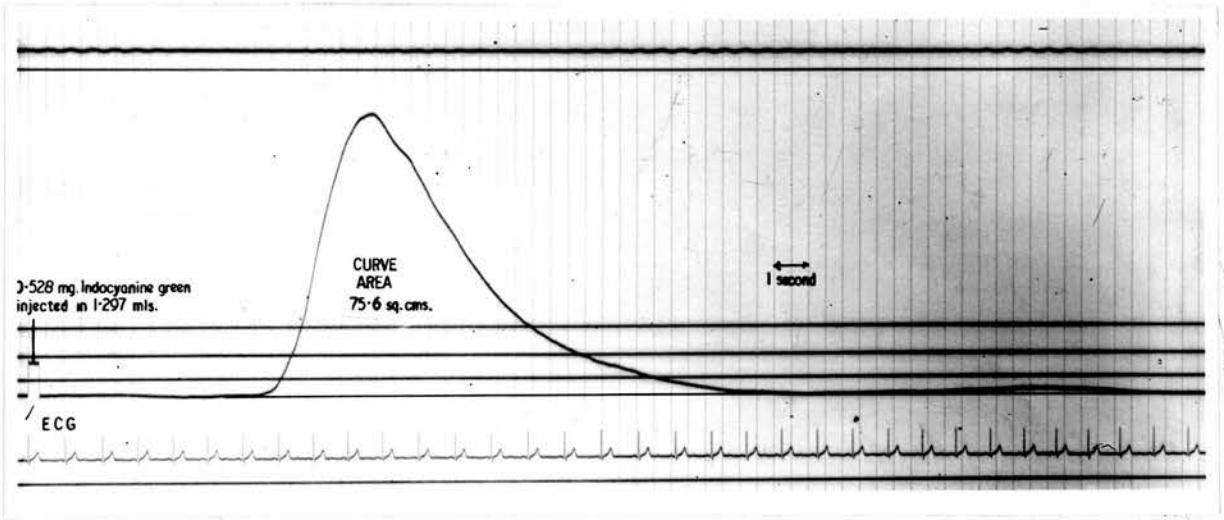

Figure 34: Unilateral renal blood flow dye-dilution curve

to baseline before recirculation occurs; curves could therefore be planimetred to determine their area without the need for a semilogarithmic replot.

Figure 35 illustrates the unilateral renal blood flow obtained during the procedure, and the equivalent cardiac output response of another subject.

Discussion

The absolutely uniform response of cardiac output in all subjects studied suggests that such a comparison between subjects is justifiable. Moreover, ideal though it may be in theory, study of arterial pressure, cardiac output and all the regional circulations simultaneously in the same subject is not possible. Several points of interest arise from these results.

The limited conclusion which may be drawn from these results is that there is a passive increase in renal blood flow with the initial rise in cardiac output, but that progressive renal vasoconstriction reduces this during the latter part of the contraction. The values for unilateral renal blood flow during the control period are approximately half the generally accepted values for total renal blood flow, suggesting that the figures obtained are measuring a flow of the same order as that through one kidney. Mention was made in a previous section that the flow often included that to structures such as the renal capsule, ureter and adrenal, and that the left spermatic vein and left adrenal vein drain into the

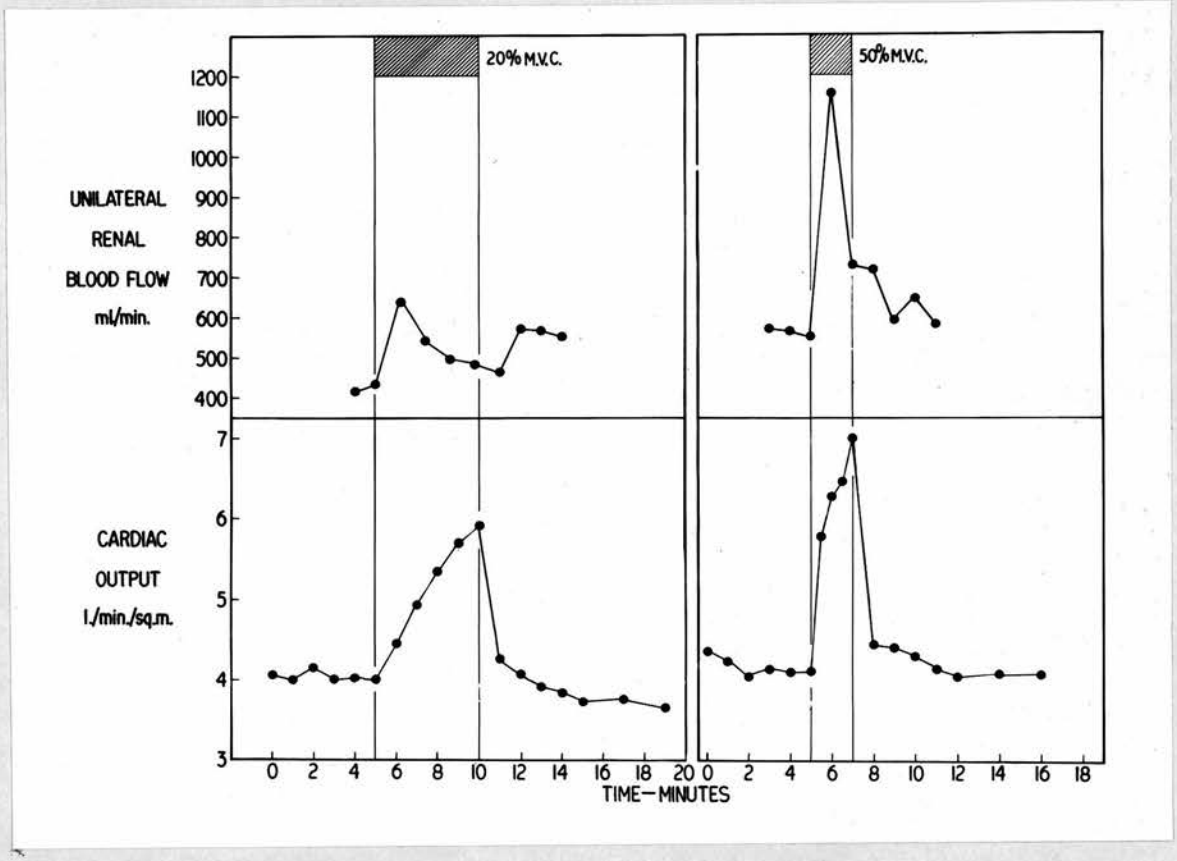

Figure 35: Response of cardiac output and renal blood flow to hand-grip

left renal vein. The anatomy of the renal artery and its distribution was shown on X-ray to be normal following the study by withdrawal of the arterial catheter into the aorta and midstream injection of radio-opaque medium. As was explained previously, the presence of multiple renal arteries may invalidate the method. Ideally, blood should also be sampled below the origin of the renal artery during injection to ensure that no dye overflows into the abdominal aorta during injection. This would require a second femoral arterial catheter which complicates the procedure. Any loss of the injected dye into the aorta, if inconstant, would give unreliable results however. The situation of the renal vein catheter was confirmed on screening, and also by blood oxygen saturation estimations. At the end of the investigation duplicate samples were analysed with the catheter in situ, and again after withdrawing it into the inferior vena cava; average values were 83 and 76 per cent. respectively.

Indocyanine green is known not to escape into the urine (Cherrick et al., 1960), and this was confirmed by spectrophotometry of the urine at the appropriate wavelength.

Although the results of this study suggest that the method is a valuable one to study rapid changes of renal blood flow in the intact subject, there are certain difficulties worth mentioning. Arteriosclerotic, kinked vessels in older subjects frequently lead to difficulties in passing the femoral artery catheter into the desired position. Without image intensification it is extremely difficult to position both

catheters satisfactorily. Several studies had to be abandoned because of repeated impaction of the sampling catheter tip on the renal vein intima, with interruption of blood withdrawal during inscription of dye curves.

In several earlier studies with this technique alternate measurements of renal blood flow and cardiac output were performed. Renal blood flow curves were obtained as described and the injection and sampling lines were then reversed so that dye was injected into the renal vein and sampled from the renal artery to obtain cardiac output curves. The concentration of dye chosen for injection gave adequate sized curves for cardiac output at sensitivity nine, and for renal blood flow at sensitivity one. Although the system worked well and adequate sized curves were obtained using the same injectate in its very different volumes of dilution by changing the sensitivity control, curves obtained across the kidney reached peak concentrations well above the linear range of the system. For alternating measurements of renal blood flow and cardiac output via the same two catheters two separate injectors are necessary, containing different dye concentrations, to ensure that the peak concentration of the curves does not exceed the upper limit of the linear range of the system. A point arising from these earlier unsuccessful studies, however, was that where renal flows and cardiac outputs were alternated at rest in an apparently stable subject the appearance times at the renal artery after renal vein injection were longer than the duration of the renal blood flow curves. This indicated that no early

recirculation was hidden within the downslope of the renal curve before it returned to baseline.

Finally, the calculated kinetic energy of injection into the renal artery with an injection volume of 1.3 ml. and an injection duration of 0.3 sec. was 8586 g. $cm.^{2}$ $sec.^{-2}$ which is below the level calculated to cause haemolysis by Andres et al. (1954).

APPENDIX II

Statistical methods were obtained from Fisher (1946) and Geigy (1956). Where Fick values are represented by 'x' and dye values by 'y', and 'n' represents the number of observations in the analysis:

$$\text{Mean } (\bar{x}) = \frac{\sum x}{n}$$

$$\text{Variance} = \frac{n\sum x^2 - (\sum x)^2}{n(n-1)}$$

$$\text{Standard deviation (S.D.)} = \sqrt{\frac{n\sum x^2 - (\sum x)^2}{n(n-1)}}$$

$$\text{Standard error of mean (S.E.)} = \sqrt{\frac{\text{Variance}}{n}}$$

$$\text{Correlation coefficient (r)} = \frac{n\sum xy - \sum x \sum y}{\sqrt{n\sum x^2 - (\sum x)^2} \times \sqrt{n\sum y^2 - (\sum y)^2}}$$

Line of regression: $y = a + bx$

$$a = \frac{\sum x \sum xy - \sum y \sum x^2}{(\sum x)^2 - n\sum x^2}$$

$$b = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x^2 - \frac{(\sum x)^2}{n}}$$

Standard error of regression coefficient (s_b) =

$$\frac{\Sigma y^2 - \bar{y}\Sigma y - b^2 (\Sigma x^2 - \bar{x} \Sigma x)}{(n - 2) (\Sigma x^2 - \bar{x} \Sigma X)}$$

Standard error of difference between regression coefficient and line of identity (s_{Db}) = $\sqrt{2 (s_b)^2}$

"Student's" t = $\frac{1 - b}{s_{Db}}$

Standard error of estimate = $\sqrt{\frac{n \Sigma y^2 - (\Sigma y)^2}{n (n - 1)}} \times (1 - r^2)$

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